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# Impacts du changement climatique sur les bilans de carbone et de gaz à effet de serre de la prairie permanente en lien avec la diversité fonctionnelle

Amélie Cantarel

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**THESE**

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**Par Amélie CANTAREL**

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**Impacts du changement climatique sur les bilans de carbone et de gaz à effet de serre de la prairie permanente en lien avec la diversité fonctionnelle**

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Impacts of climate change drivers on grassland structure, production and greenhouse gas fluxes

Composition du Jury :

Paul LEADLEY <i>Professeur, Université Paris Sud 11, France</i>	Rapporteur
Andreas LÜSCHER <i>Directeur de Recherche, Agroscope, Suisse</i>	Rapporteur
Catherine HENAULT <i>Directeur de Recherche, INRA Orléans, France</i>	Examineur
Marie-Pascale PRUD'HOMME <i>Professeur, Université Caen, France</i>	Examineur
Patricia DREVET-ROECKEL <i>Professeur, Université Blaise Pascal Clermont-Ferrand, France</i>	Correspondant Universitaire
Juliette BLOOR <i>Chargé de Recherche, INRA Clermont-Ferrand, France</i>	Co-encadrante de Thèse
Jean-François SOUSSANA <i>Directeur de Recherche, DS INRA Paris, France</i>	Directeur de Thèse

Laboratoire d'accueil : INRA – Unité de Recherche sur l'Ecosystème Prairial (UR 874)  
234 Avenue du Brézet – 63100 Clermont-Ferrand



# Résumé

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En Europe, la prairie occupe près de 40% de la surface agricole utile et fournit un ensemble de services environnementaux et agricoles, tout en constituant un réservoir de diversité végétale et animale. Cet écosystème herbacé, plurispécifique et multifonctionnel est un système biologique complexe qui fait interagir l'atmosphère, la végétation et le sol, via les cycles biogéochimiques, notamment ceux du carbone et de l'azote. Motivées par le maintien des biens et services des prairies face aux changements climatiques et atmosphériques, les recherches actuelles sur l'écosystème prairial s'attachent à étudier l'évolution des processus clés du système prairial (i.e. production, échanges gazeux, changements d'espèce) sous changement climatique complexe.

Ce projet de thèse a pour objectif d'étudier *in situ* les impacts des principales composantes du changement climatique (température de l'air, précipitations, concentration atmosphérique en gaz carbonique) sur des prairies extensives de moyenne montagne. Nous cherchons à mettre en évidence les changements de structure et de fonctionnement de l'écosystème prairial sous l'influence d'un scénario de changement climatique prévu à l'horizon 2080 pour le centre de la France. Ce scénario (ACCACIA A2) prévoit une augmentation de 3.5°C des températures de l'air, une augmentation des concentrations atmosphériques en CO<sub>2</sub> de 200 ppm et une réduction des précipitations estivales de 20 %.

Nos résultats indiquent qu'à moyen terme (trois ans de traitements expérimentaux) le réchauffement a des effets néfastes sur la production annuelle du couvert végétal. L'effet bénéfique d'une élévation des teneurs en CO<sub>2</sub> sur la production aérienne n'apparaît qu'à partir de la troisième année. La richesse spécifique (nombre d'espèces) et les indices de diversité taxonomique n'ont pas montré de variations significatives sous changement climatique. Cependant après trois années de réchauffement, l'abondance des graminées semble être altérée. Contrairement à la production, les traits sont plus affectés par la concentration en CO<sub>2</sub> élevée que par le réchauffement. Après trois ans de traitements, des mesures d'échanges gazeux (CO<sub>2</sub>) à l'échelle du couvert végétal pendant la saison de croissance ont montré un effet négatif du réchauffement sur l'activité photosynthétique du couvert et une acclimatation de la photosynthèse au cours de la saison de croissance sous CO<sub>2</sub> élevé. Ces tendances ont aussi été trouvées sur la photosynthèse foliaire d'une des espèces dominantes du couvert (*Festuca arundinacea*). L'effet négatif direct du réchauffement à l'échelle foliaire semble être associé à une diminution des sucres dans les limbes. L'acclimatation à l'enrichissement en CO<sub>2</sub> à l'échelle foliaire, quant à elle, semble être indirectement dépendante du statu hydrique du sol. Notre étude a aussi porté sur l'analyse des échanges gazeux sol-atmosphère d'un des principaux gaz à effet de serre trace des prairies, l'oxyde nitreux (N<sub>2</sub>O). Malgré une forte variabilité inter- et intra-annuelle, les flux de N<sub>2</sub>O semblent être favorisés sous réchauffement. L'augmentation de la température affecte aussi positivement les taux de nitrification et leur pool microbien associé (AOB), et les rejets de N<sub>2</sub>O via dénitrification. De plus, les flux de N<sub>2</sub>O mesurés aux champs ont montré une corrélation plus forte à la taille des populations microbiennes (nitrifiantes et dénitrifiantes) en traitement réchauffé qu'en traitement témoin. En conclusion, la température semble être le facteur principal dans les réponses de cette prairie aux changements climatiques futurs. De plus, nos résultats suggèrent que le fonctionnement (production, émissions de N<sub>2</sub>O) des prairies extensives de moyenne montagne est plus vulnérable aux changements climatiques que la structure de la communauté végétale.

**Mots-clés :** prairies permanentes, changement climatique, biomasse aérienne, traits fonctionnels, échanges gazeux en CO<sub>2</sub>, flux de N<sub>2</sub>O

# Abstract

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In France, the grassland ecosystem represents an important part of the total of agricultural landscape and provides important economic and ecological services. This multifunctional ecosystem is a complex biological system where atmosphere, plants and soil interact together, *via* the biogeochemical cycles (particularly carbon and nitrogen cycles). In order to maintain goods and services from grasslands in changing environmental conditions, current research on the grassland ecosystem focus on the evolution of key grassland processes (i.e. production, gaseous exchanges, biodiversity) under multiple and simultaneous climate change.

This thesis addresses the impacts of the three main climate change drivers (air temperature, precipitation and atmospheric carbon dioxide concentrations) on an extensively-managed upland grassland *in situ*. We investigated changes in ecosystem function and structure under the influence of a projected climate scenario for 2080 for central France. This scenario (ACCACIA A2) comprises: air warming of 3.5°C, 20 % reduction of the summer precipitation and an increase of 200 ppm in atmospheric carbon dioxide (CO<sub>2</sub>).

Our results indicate that in the medium term (after three years of experimental treatments), warming had negative effects on the annual aboveground production. Elevated CO<sub>2</sub> had no significant effects on aboveground production initially, but positive effects on biomass from the third year onwards. Species richness and the indices of species diversity did not show significant differences in response to climate change, but warming was associated with a decline in grass abundance after three years. Contrary to biomass production, plant traits showed a stronger response to elevated CO<sub>2</sub> than to warming. After three years of study, canopy-level photosynthesis showed a negative effect of warming but an acclimation to elevated CO<sub>2</sub> during the growing season. This pattern was also found for leaf-level photosynthetic rates measured on a dominant grass species (*Festuca arundinacea*). For *Festuca*, the direct negative effect of warming was associated with a decrease in leaf fructan metabolism. In contrast, the photosynthetic acclimation under elevated CO<sub>2</sub> observed in *Festuca* seemed closely linked to the indirect effect of soil water content. Our study also examined effects of climate change on one of the main trace greenhouse gases in grasslands, nitrous oxide (N<sub>2</sub>O). During our study, N<sub>2</sub>O fluxes showed significant inter-and intra-annual variability. Nevertheless, mean annual N<sub>2</sub>O fluxes increased in response to warming. Warming had a positive effect on nitrification rates, denitrification rates and the population size of nitrifying bacteria (AOB). Furthermore, field N<sub>2</sub>O fluxes showed a stronger correlation with the microbial population size in the warmed compared with the control treatment. Overall, warming seems to be the main factor driving ecosystem responses to projected climate change conditions for this cool, upland grassland. In addition, our results suggest that grassland function (aboveground production, N<sub>2</sub>O emissions) are more vulnerable to complex climate change than grassland community structure for our study system.

**Key words:** grassland, climate change, aboveground biomass, plant traits, CO<sub>2</sub> gaseous exchange, N<sub>2</sub>O fluxes

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*" Nos vérités sont provisoires : battues en brèche par les vérités de demain, elles s'embroussaillent de tant de faits contradictoires que le dernier mot du savoir est le doute. "*

*Jean-Henri Fabre, Souvenirs entomologiques (1870-1889)*





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# ***Introduction***

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L'impact des changements climatiques sur le fonctionnement et la structure des communautés végétales fait l'objet de préoccupations importantes, du fait de ses conséquences sur la biodiversité et les cycles biogéochimiques. Les expériences de manipulation des écosystèmes permettent de mieux comprendre les processus biotiques (processus liés aux organismes vivants) et abiotiques (processus physico-chimiques) en réponses à des forçages climatiques et d'améliorer les modèles utilisés pour évaluer les impacts du changement climatique (Norby & Luo 2004). Le changement climatique modifiant plusieurs variables environnementales (température, précipitations, concentration atmosphérique en gaz carbonique), la mise en place d'expérimentations multifactorielles se révèle être primordiale afin de mieux comprendre leurs effets, directs et indirects, sur l'écosystème et de les quantifier.

Ce travail de thèse cherche à mettre en évidence les interactions entre les changements de fonctionnement de l'écosystème (*via* la production, les traits végétaux, ou encore les activités microbiennes) et ceux des cycles biogéochimiques carbone (C) – azote (N) (*via* les flux de dioxyde de carbone, CO<sub>2</sub> et d'oxyde nitreux, N<sub>2</sub>O) sous l'influence d'un scénario expérimental de changement climatique. Le cadre conceptuel de la thèse repose sur les liens entre fonctionnement et structure de l'écosystème suite à un changement de conditions environnementales, et met en avant la présence d'effets directs sur les cycles biogéochimiques et d'effets indirects *via* des changements de diversités spécifique (microbiennes ou végétales) et fonctionnelle. Cette partie introductive fait un point sur l'état des connaissances existantes sur ce sujet et situe le contexte de la thèse avant de définir la démarche expérimentale mise en œuvre.



# 1- Le changement climatique, scénarios et impacts

## 1.1 Les écosystèmes terrestres dans un environnement changeant

Les écosystèmes terrestres répondent aux fluctuations des variables biotiques et abiotiques. Les changements quotidiens et saisonniers de la lumière, de la température et de l'humidité sont les caractéristiques évidentes de n'importe quel environnement naturel (Norby & Luo 2004). Les contraintes biotiques, telles que les maladies, ou encore les ravageurs (*i.e.* insectes) peuvent également altérer la structure des écosystèmes (Ricklefs & Miller 2005). A cette suite complexe de perturbations naturelles s'ajoutent des modifications rapides du système climatique (GIEC 2001, 2007). En effet, si certains aspects du changement climatique actuel peuvent passer pour des éléments de variabilité de fond ou montrent une tendance évolutive régulière, d'autres sont plus nouveaux et pour le moins inhabituel (GIEC 2007). A la variabilité interne du système climatique et des facteurs extérieurs naturels, s'ajoute l'impact des activités anthropiques et leurs conséquences sur le système climatique. Actuellement, la communauté scientifique admet que le changement climatique observé ces dernières décennies est très probablement le fait d'une augmentation de la concentration en dioxyde de carbone (CO<sub>2</sub>) et autres gaz à effet de serre traces (GES<sup>1</sup>) dans l'atmosphère, à laquelle les activités humaines ont très fortement contribué (Figure 1). Les travaux du GIEC (2001) montrent que depuis 1750, la concentration atmosphérique en CO<sub>2</sub> s'est accrue d'un tiers, passant de 280 ppm<sup>2</sup> avant la révolution industrielle à 380 ppm à ce jour (GIEC 2007). Cette évolution des concentrations en GES a induit une augmentation de la température moyenne globale à la surface du globe, entraînant également des changements dans la distribution spatio-temporelle des précipitations (GIEC 2001, 2007).

Dans cette première partie, nous présenterons les scénarios de prédictions climatiques futures et le type d'approches utilisables pour améliorer l'état des connaissances sur les réponses des écosystèmes au changement climatique prédit. Afin de faciliter la lecture de ce manuscrit, le terme de changement climatique sera utilisé *sensu largo* dans le but d'inclure à

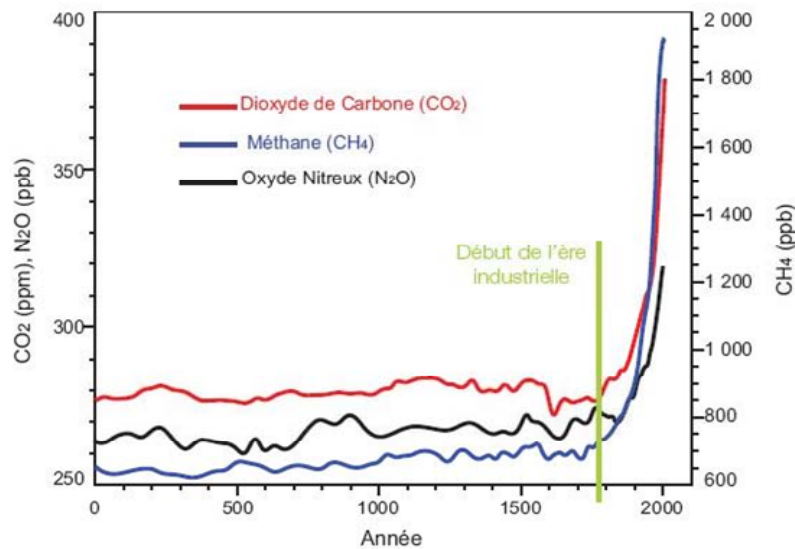
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<sup>1</sup> Gaz à effets de serre (CO<sub>2</sub>) et principaux gaz à effets de serre traces : méthane (CH<sub>4</sub>) et oxyde nitreux (N<sub>2</sub>O)

<sup>2</sup> ppm : (parties par million) désigne le rapport du nombre de molécules de gaz à effet de serre au nombre de molécules d'air sec. 300ppm signifient 300 molécules de gaz à effet de serre par million de molécules d'air sec.



la fois les modifications du climat (élévation de la température et modifications de la distribution spatio-temporelle des précipitations) et l'augmentation de la concentration atmosphérique en CO<sub>2</sub>.

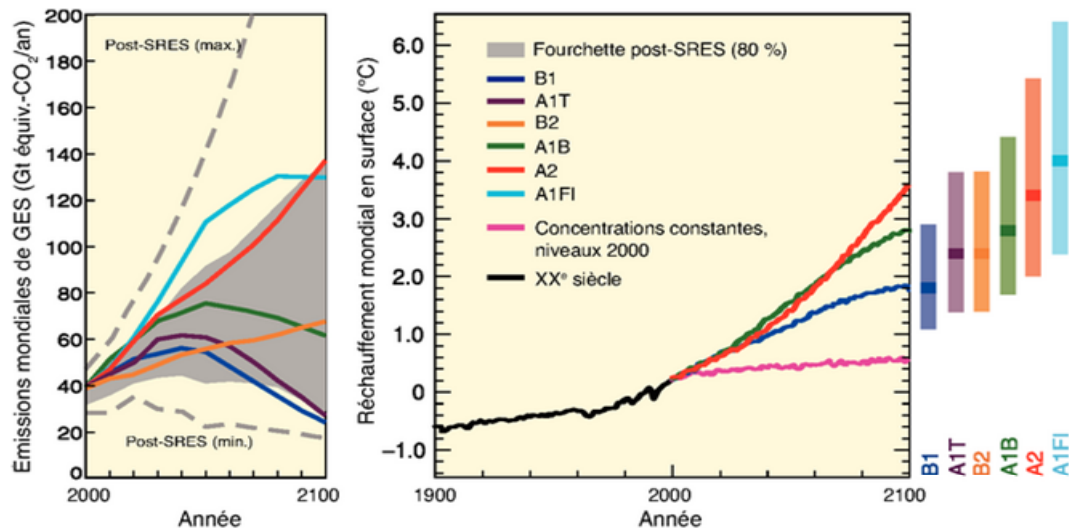


*Figure 1 : Evolution de la concentration des gaz à effet de serre (CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O) au cours des 10 000 dernières années (d'après le GIEC 2007). Les données ont été obtenues à partir de carottes de glace et de mesures récentes.*

## 1.2 Le changement climatique, quelles prédictions pour le futur ?

Pour la fin du XXI<sup>ème</sup> siècle, les modèles climatiques développés par le GIEC montrent un accroissement du [CO<sub>2</sub>] pour atteindre des valeurs situées entre 540 et 970 ppm (GIEC 2007). L'accroissement moyen de la température de surface entre 1990 et 2100 est estimé entre 1.5 et 6°C (Figure 2), et la gamme de réchauffement en fonction des scénarios d'émissions de gaz à effet de serre va de 1.5°C (avec une fourchette de vraisemblance de 1.1 à 2.9) à 4.0°C (fourchette de 2.2 à 6.4). Une comparaison des plus récents scénarios d'évolution de la pluviométrie saisonnière dans 32 régions du monde, faite par le groupe II du GIEC, montre une tendance à l'augmentation pour l'Europe du Nord (0 à + 3 % par décennie) au printemps, à l'automne et en hiver. En revanche, pour la zone Europe du Sud, les modèles prédisent une réduction de la pluviométrie estivale (de -0.2 à -6 % par décennie). Parmi les conclusions retenue par le GIEC concernant l'évolution des événements climatiques extrêmes, les plus probables (à plus de 95%), sont celles d'une augmentation des températures maximales et

minimales couplée respectivement à un accroissement de la fréquence des jours chauds et une diminution de celle des jours froids (ou des gelées).

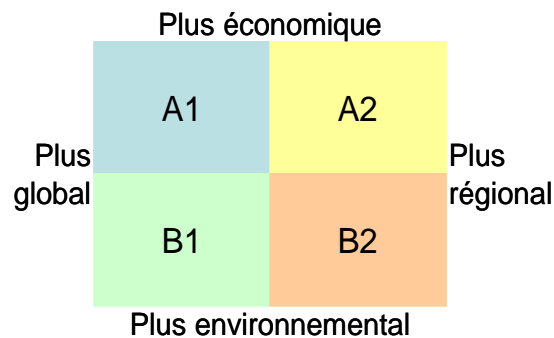


**Figure 2 : Prévisions de l'évolution des émissions de GES (en Gt équiv. CO<sub>2</sub>) (à gauche) et de l'évolution des températures (à droite) selon différents scénarios climatiques d'ici 2100. Les barres sur la droite précisent la valeur la plus probable (zone foncée) et la fourchette probable correspondant aux six scénarios de référence du SRES pour la période 2090-2099. Tous les écarts de température sont calculés par rapport à 1980-1999 (d'après le GIEC 2007).**

Le GIEC a élaboré, et fait évoluer, depuis sa création (1987) des scénarios concernant l'évolution future des émissions de gaz à effet de serre, des températures et de la distribution spatio-temporelle des précipitations. Le *Special Report on Emissions Scenarios* (SRES ; Nakicenovic *et al.* 2000 ; GIEC 2001) propose quatre grandes familles de scénarios de références notées successivement A1, A2, B1 et B2 (Nakicenovic *et al.* 2000). Ces scénarios reposent sur différentes hypothèses (Figure 3) relatives à la croissance démographique et économique, l'exploitation des sols, les progrès technologiques et l'approvisionnement énergétique, ainsi qu'à la façon dont les différentes sources d'énergie contribueront à cet approvisionnement entre 1990 et 2100 (GIEC 2001, 2007). Suivant les hypothèses de construction retenues, les scénarios ne vont pas donner les mêmes évolutions, ni avoir le même impact climatique (exemple pour les températures et les émissions de GES, Figure 2). Les 4 scénarios SRES ont été caractérisés pour l'Europe, lors du projet Européen ACACIA<sup>3</sup> sur plusieurs périodes temporelles, à l'horizon 2020, 2050 et 2080. Ces modèles climatiques

<sup>3</sup> ACACIA : A Concerted Action towards a Comprehensive climate Impacts and Adaptations assessment

peuvent être alors utilisés pour prédire l'évolution future du climat à l'échelle de l'Europe, de la France, ou avec des incertitudes croissantes à l'échelle de petites régions.



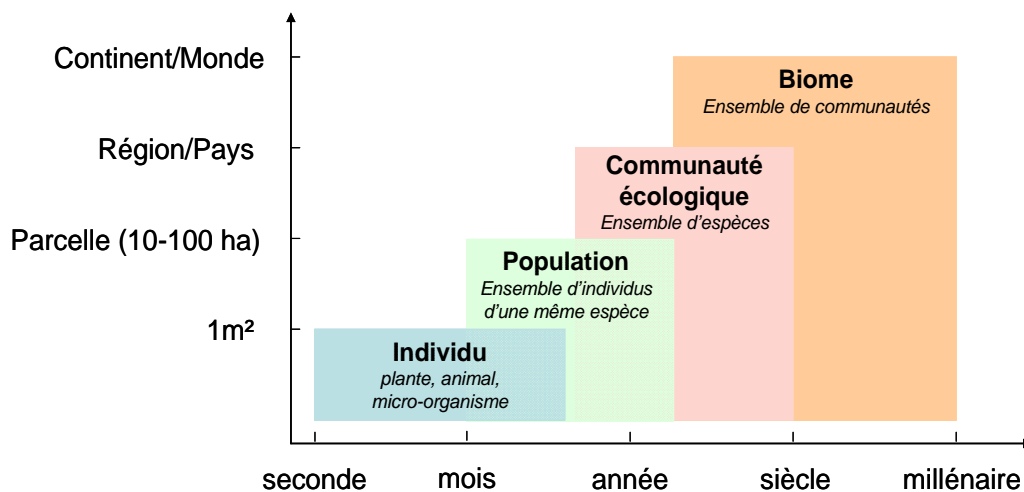
*Figure 3 : Représentation schématique des scénarios SRES. Les familles de scénario A1 et A2 intègrent une vision plus économique que les scénarios B1 et B2 qui eux sont environnementalistes. A1 et B1 sont plus globaux comparés à A2 et B2 qui sont régionaux (d'après le GIEC 2001)*

### **1.3 Impacts biologiques et écologiques du changement climatique : niveaux d'organisation et échelles spatiales et temporelles**

Le changement climatique implique des augmentations simultanées de la teneur en CO<sub>2</sub> atmosphérique et de la température de l'atmosphère, couplées à des modifications des précipitations (Weltzin & Tissue 2003). Ces trois facteurs interviennent dans la régulation de processus biologiques et physico-chimiques à des échelles emboîtées allant de l'organisme jusqu'à l'écosystème (Beier 2004). Dès lors, le changement climatique est susceptible d'avoir des impacts majeurs sur la structure, la dynamique et le fonctionnement des écosystèmes terrestres (Norby & Luo 2004). Ce phénomène pose la question du maintien des écosystèmes, de leur biodiversité et de leurs services au cours des décennies à venir (Rockström *et al.* 2009).

Les impacts du changement climatique sur les écosystèmes sont complexes car ils impliquent des interactions entre les échelles temporelles et spatiales (Figure 4 ; GIEC 2007), les temps de réponses étant déterminés par l'échelle spatiale ou niveau d'organisation considéré(e). Par exemple, les temps de réponse d'ordres physiologiques, à l'échelle de l'individu sont rapides, de l'échelle de la seconde à celle du mois et opèrent à de petites échelles spatiales (de la cellule au m<sup>2</sup>). Ils sont pourtant à l'origine de la modification des populations et des communautés en réponse aux conditions environnementales, tant à

l'échelle de la parcelle que celle de la région. Les réponses des populations (ensemble d'individus de la même espèce) interviennent par conséquent à des échelles temporelles intermédiaires (*i.e.* du mois au siècle) (Figure 4) et sous-tendent des changements de biodiversité, ceux-ci pouvant inclure des changements au niveau génétique de nature adaptative (*i.e.* démontré pour les arbres, Jump *et al.* 2006 ; et les coraux, Coles & Brown 2003). Les groupements d'espèces ou communautés écologiques (*i.e.* écosystèmes) ont des temps de réponse de l'année au siècle opérant à l'échelle régionale. Et enfin, à l'échelle continentale, les biomes (ensemble d'écosystèmes) présentent les temps de réponse au changement climatique les plus longs, partant de la décennie au millénaire (*i.e.* Davis 1989; Prentice *et al.* 1991; Lischke *et al.* 2002; Neilson *et al.* 2005).



**Figure 4 : Illustrations des interactions entre échelles spatiales et temporelles au niveau desquelles le changement climatique peut agir (d'après le GIEC 2007)**

Dans ce travail de thèse, le niveau d'organisation retenu est celui de l'écosystème ou communauté écologique (Figure 4) comprenant les populations végétales et les microorganismes du sol associés. Nous considérons de petites échelles spatio-temporelles (de l'ordre du mètre carré et de la décennie). Les processus étudiés correspondent donc à des processus assez rapides (dynamique des communautés ou des populations) et locaux. La démarche adoptée devrait donc permettre de progresser dans la compréhension des mécanismes agissant à court et moyen termes et susceptibles d'entraîner des modifications de dynamique et de fonctionnement des écosystèmes à des échelles spatiales plus grandes sur le long terme.

## 1.4 Méthodes d'étude des impacts du changement climatique sur les écosystèmes

L'évaluation des impacts du changement climatique sur les écosystèmes a été réalisée jusqu'à présent soit directement *via* des approches expérimentales (Wright *et al.* 1998, Beier 2004, Canadell *et al.* 2007), soit indirectement *via* modélisation (Riedo *et al.* 1998 ; Thornley & Cannell 2000).

### 1.4.1 Expérimentation et modélisation, complémentarité des méthodes

Malgré la complexité et la variabilité spatiale et temporelle des processus au sein d'un écosystème, les expériences de manipulations au champ permettent l'étude des processus à plusieurs niveaux d'échelle (*i.e.* espèce, communauté, population, écosystème) et sont devenues un outil important pour améliorer l'état des connaissances sur les processus écosystémiques et leurs réponses aux stress environnementaux (Beier 2004). En outre, les expériences manipulant des écosystèmes sont uniques pour valider et tester les modèles simulant les impacts du changement climatiques. Les expériences à l'échelle des écosystèmes sont des outils précieux pour fournir des informations intégratives au niveau des processus biologiques (Norby & Luo 2004). Ainsi à la fin des années 1980, après une dizaine d'années de recherches expérimentales sur les réponses des principaux écosystèmes terrestres (prairies, grandes cultures et forêts) à une augmentation du CO<sub>2</sub> atmosphérique (*i.e.* en condition contrôlée), de nouvelles techniques d'enrichissement en CO<sub>2</sub> de l'atmosphère des systèmes considérés ont vu le jour. L'actuelle technique de référence (FACE, 'Free Air Carbon dioxide Enrichment') permet de réaliser une fumigation contrôlée en CO<sub>2</sub> à l'air libre et à l'échelle d'un écosystème (Miglietta *et al.* 2001). D'autres méthodes, en serre ou à l'air libre, ont également été proposées pour réchauffer des écosystèmes et pour modifier les précipitations. L'augmentation de la température peut se faire soit de manière passive (*i.e.* réchauffement en utilisant un gradient altitudinal, Bloor *et al.* 2010 ; réchauffement passif de nuit grâce à des volets roulants, Mikkelsen *et al.* 2007), soit de manière active (*i.e.* réchauffement par radiateurs infrarouges, Nijs *et al.* 1996). Quant aux modifications des précipitations, la méthode la plus fréquente est celle qui consiste à intercepter les pluies (écrans pluies manuels ou automatisés, Mikkelsen *et al.* 2007) et à gérer les apports en eau par la suite.

L'approche expérimentale couplée à l'approche par modélisation, mécaniste ou stochastique, permet de formaliser les flux de données et de connaissances (combinant échelles spatiales et temporelles), étape nécessaire à la simplification et à la compréhension des lois d'actions régissant le fonctionnement d'un système. Quand la modélisation se veut prédictive ou prospective, elle permet de prévoir les réponses (*i.e.* dynamiques de production, de distribution d'espèces, etc.) d'un système à la variation des facteurs du milieu (abiotiques et biotiques) et de décrire des situations futures. La modélisation nécessite généralement d'être calée par des vérifications "*in situ*", lesquelles passent par la paramétrisation et le calibrage des « modèles » utilisés. Il est donc important que le développement de modèles de plus en plus complexes, aille de pair avec un progrès dans les approches expérimentales (Körner *et al.* 2007). Depuis une vingtaine d'années, de nombreuses études ont tenté de modéliser numériquement les impacts du changement climatique sur la production agricole (Gitay *et al.* 2001). Cette démarche repose sur le couplage entre modèles du climat et modèles d'écosystèmes (Riedo *et al.* 1998 ; Thornley & Cannell 2000). Beaucoup de modèles d'écosystèmes ont ainsi été développés (*i.e.* Parton *et al.* 1987; Comins & McMurtrie 1993; Rastetter *et al.* 1997; Sitch *et al.* 2003), validés par des résultats expérimentaux et appliqués pour examiner les réponses des écosystèmes au changement climatique (Cramer *et al.* 2001; Luo *et al.* 2001; Hanson *et al.* 2005). La modélisation peut alors aider à extrapoler les résultats expérimentaux de quelques sites modèles à d'autres écosystèmes, voire à des secteurs géographiques et des échelles temporelles plus larges (Luo *et al.* 2008).

#### **1.4.2 L'expérimentation, outil d'étude de l'impact du changement climatique sur les écosystèmes**

Dans le contexte actuel du changement climatique, il est nécessaire de développer une meilleure compréhension des réponses adaptatives des écosystèmes : i) aux modifications tendanciennes des variables climatiques et atmosphériques (élévation de la concentration atmosphérique en CO<sub>2</sub>, réchauffement, variations de la pluviométrie) puis ii) à l'augmentation de la variabilité du climat et de la fréquence des événements climatiques extrêmes (Tubiello *et al.* 2007). Les uns pouvant moduler les effets des autres, les interactions (antagonistes ou en synergie) entre les différents facteurs doivent être analysées et comprises.

Afin de prendre en compte la complexité du changement climatique, de nombreux

projets de recherche, impliquant souvent des études mono-factorielles, conduites à différentes échelles d'organisation biologique (*i.e.* de la plante isolée en environnement contrôlé, jusqu'à l'échelle de l'écosystème étudié *in situ*) ont été réalisés. Pendant les deux dernières décennies, plusieurs expériences *in situ* ont examiné les effets de facteurs du changement climatique et atmosphérique (élévation du CO<sub>2</sub> atmosphérique ou de la température ou variation de la pluviométrie) de manière disjointe, sur différents écosystèmes (forêts, prairies, cultures, etc.) avec des durées d'expérimentation souvent trop courtes pour pouvoir évaluer son impact à long terme ( $\leq 4$  ans ; *i.e.* Owensby *et al.* 1993, Potvin & Vasseur 1997, Teyssonneyre *et al.* 2002 pour le CO<sub>2</sub> ; Sternberg *et al.* 1999, Fay *et al.* 2003 pour la pluviométrie ; ou encore Price & Vasseur 2000, de Boeck *et al.* 2008 pour le réchauffement). Or le changement climatique est par essence un phénomène multifactoriel agissant à moyen et long terme, notamment en milieu prairial. La mise en place d'expérimentations *in situ* mettant en interaction les 3 composantes du changement climatique, augmentation du CO<sub>2</sub> atmosphérique, augmentation de la température et réduction de la pluviométrie, se révèle être primordiale. Jusqu'à présent, peu d'expériences multifactorielles manipulant des variables de changement climatique ont été réalisées à l'échelle d'écosystèmes entiers. Le projet de CLIMEX<sup>4</sup> conduit en Norvège dans les années 1995-1999 (Wright *et al.* 1998) fut la première expérience de changement climatique dans laquelle un écosystème forestier boréal complet a été exposé simultanément à une élévation des teneurs en CO<sub>2</sub> et des températures. Plus récemment (en 1998), une expérience de changement climatique portant sur des prairies méditerranéennes à Jasper Ridge (JRGE<sup>5</sup>, Californie, USA) a combiné les effets d'une augmentation des teneurs en CO<sub>2</sub>, des températures, de la pluviométrie et de la déposition en azote atmosphérique. Cette étude a clairement montré la complexité des réponses d'un écosystème modèle dans un monde 'multifactoriel' (Canadell *et al.* 2007). Ces essais ont également mis en évidence la nécessité d'avoir des références expérimentales sur un plus grand nombre d'écosystèmes et de contextes pédo-climatiques.

Pour ce travail de thèse, nous nous sommes appuyés sur un dispositif multifactoriel *in situ* à l'échelle de l'écosystème afin d'étudier l'impact d'un scénario modifiant plusieurs composantes climatiques (*i.e.* température, précipitations, concentration en CO<sub>2</sub>

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<sup>4</sup> CLIMEX : CLIMate EXPeriment

<sup>5</sup> JRGE : Jasper Ridge Global Change Experiment

atmosphérique) sur la structure et le fonctionnement de l'écosystème prairial.

## **2. Quels impacts du changement climatiques sur l'écosystème prairial ?**

L'objet est ici de faire un état des connaissances sur la problématique des effets du changement climatique sur un écosystème modèle : la prairie. Nous aborderons en premier lieu l'importance des enjeux et des services écosystémiques rendus liés au maintien des prairies. Dans un second temps, nous analyserons les résultats issus de la bibliographie sur l'impact des différents facteurs climatiques sur le fonctionnement et la structure des prairies.

### **2.1 Services des écosystèmes prairiaux**

Les écosystèmes produisent des services et des biens à l'Humanité qui sont nécessaires à sa survie (i.e nourriture, des matières premières, énergie, eau) (Daily & Matson 2008). Ces services ont été classés par le Millenium Ecosystem Assessment (2005) en plusieurs catégories : services de support, de fourniture, de régulation et culturels. La figure 5 représente l'intensité des liens les plus courants entre les catégories de services d'origine écosystémique et les composantes du bien-être de l'Homme. L'intensité des liens et le potentiel de médiation diffèrent suivant les écosystèmes et les régions (Figure 5). En plus de l'influence des services d'origine écosystémique sur le bien-être de l'Homme d'autres facteurs aussi bien environnementaux qu'économiques, sociaux, technologiques et culturels jouent sur ce bien-être humain, et les écosystèmes sont à leur tour affectés par les modifications du niveau de bien-être humain. Le maintien du flux de ces biens et services résulte du bon fonctionnement des écosystèmes et de la préservation de leur biodiversité.

Pour répondre aux questions que suscite le changement climatique, nous avons choisi l'écosystème prairial comme objet d'étude de cette thèse, et plus particulièrement la prairie permanente de moyenne montagne. En effet, les prairies constituent l'un des principaux biomes à l'échelle mondiale<sup>6</sup>. Les prairies tempérées représentent, quant à elles, environ 9 millions de km<sup>2</sup> (Schlesinger 1991) et sont un écosystème aux valeurs sociétales et

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<sup>6</sup> 40.5 % de la surface terrestre en excluant l'Antarctique et le Groenland, FAO 2005



écologiques fortes de part l'importance du devenir de sa production dans le secteur agricole (*i.e.* fourrages) et de sa diversité d'espèces. D'un point de vue scientifique, l'écosystème prairial est un système réactif à des pas de temps relativement courts et facilement manipulable car composé d'espèces herbacées à croissance rapide (Fischer *et al.* 2008).

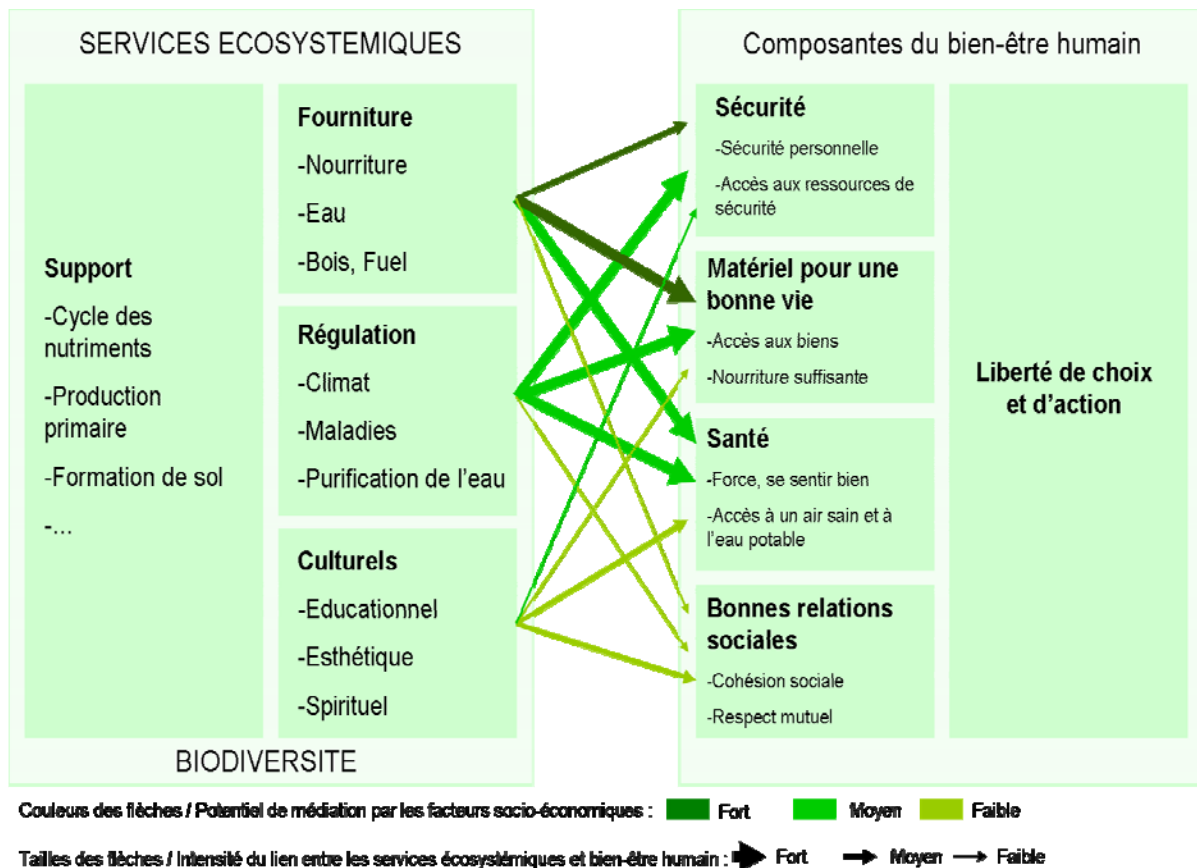
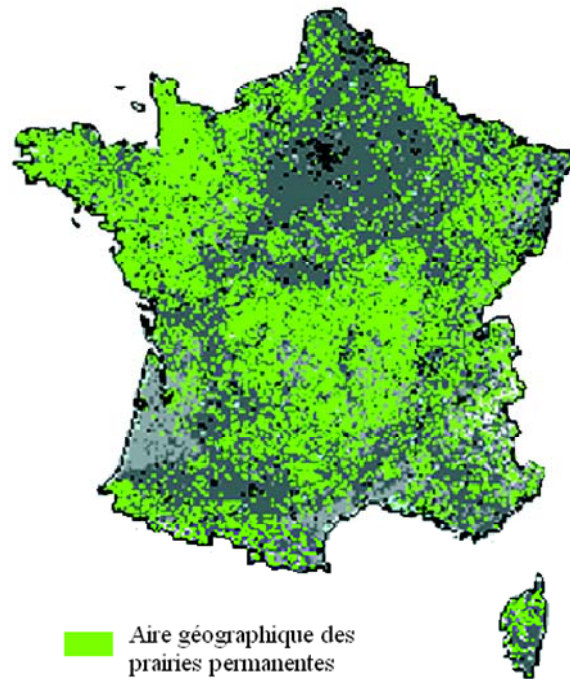


Figure 5 : Liens entre services d'origine écosystémique et les composantes du bien-être de l'Homme (d'après le Millenium Assessment 2005)

En France, la prairie constitue une formation végétale majeure puisqu'elle occupe près de 25% de la superficie du territoire national, contre 20% en moyenne en Europe (EEA, 2005). La prairie permanente française représente 80% de cette superficie (Figure 6; Agreste 2010). Les prairies permanentes (en opposition aux prairies temporaires et artificielles) sont des formations végétales maintenues au stade herbacé par le pâturage et la fauche pour l'alimentation des bovins, ovins et caprins. Les atouts des prairies portent sur la capacité à remplir des services liés au maintien de leur biodiversité (Briemle *et al.* 1997 ; Zdanowicz *et al.* 2005) qu'il s'agisse de services de fourniture (production fourragère pour l'alimentation des ruminants, biomasse pour production de bio-carburants), de support (maintien de la

fertilité des sols, Cossée 1999 ; protection des sols contre l'érosion, Briemle & Elsässer 1997), de régulation (de la qualité des eaux de surfaces et du sol, Briemle & Elsässer 1997 ; du climat *via* la séquestration du carbone dans les sols, (Soussana *et al.* 2007, 2010), mais aussi des services culturels (qualité, aménités paysagères, Ostermann, 1998).



**Figure 6 : Distribution des prairies permanentes sur le territoire français (d'après l'Agence Environnementale Européenne (EEA) 2005)**

Les enjeux écosystémiques des prairies permanentes doivent impliquer une approche multifonctionnelle de ces surfaces intégrant les dimensions économiques (réduction des coûts de production, sécurisation des systèmes fourragers, autonomie fourragère des exploitations, maintien de la fertilité des sols) mais aussi environnementales (conservation de la qualité de l'environnement : eau, sols et biodiversité, changements climatiques et contribution nette à l'effet de serre) et écologiques ou paysagères (maintien de la biodiversité, entretien des paysages ruraux, Hervieu 2002). Pour comprendre comment l'écosystème prairial répond au changement climatique, nous avons choisi de nous intéresser à trois types de processus écosystémiques : la production de biomasse aérienne, les cycles C-N et les flux de gaz à effet de serre (GES) associés, puis la diversité des prairies

## 2.2 Fonctionnement et diversité des écosystèmes prairiaux

### 2.2.1 Production de biomasse aérienne et productivité primaire

En présence de lumière, les plantes chlorophylliennes fixent le  $\text{CO}_2$  atmosphérique dans leur tissu par le processus de photosynthèse, catalysée par l'activité carboxylase de la Rubisco. Il en résulte la synthèse de sucres simples et la production d'oxygène et la consommation d'eau (Emerson & Arnold 1932). La photosynthèse est pilotée par l'ouverture des stomates qui régulent les échanges gazeux au sein de la feuille. Lorsqu'ils sont saturés en eau, les stomates s'ouvrent et permettent alors les échanges gazeux de la plante avec l'atmosphère (Schulze 1986). En absence de lumière, les plantes libèrent dans l'atmosphère du  $\text{CO}_2$  par autotrophie<sup>7</sup>. Une partie du carbone fixé par les plantes va retourner au sol *via* la décomposition des racines et de la litière végétale (5 à 10 %, Davet 1996). Ce carbone est la principale source d'énergie pour les communautés des microorganismes hétérotrophes<sup>8</sup> (Figure 7).

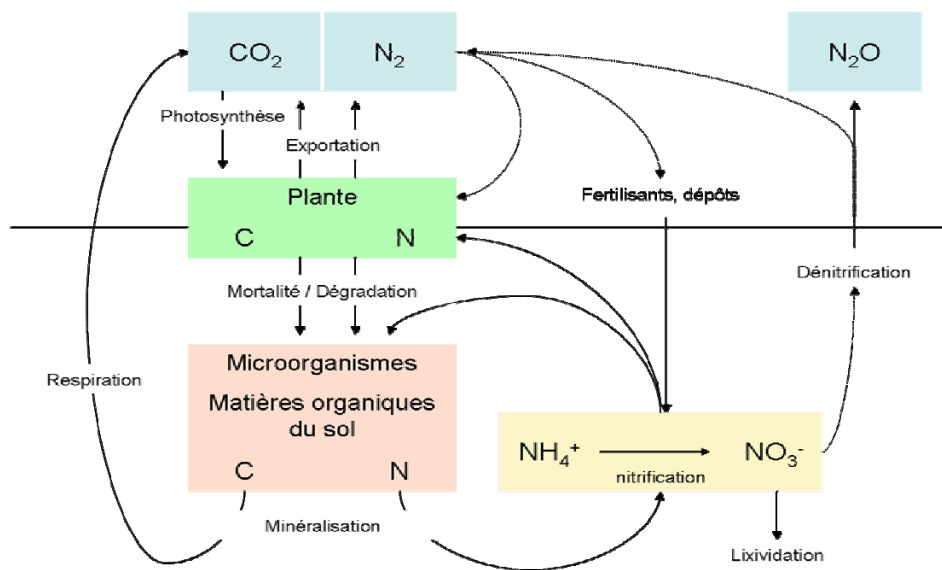


Figure 7 : Cycles couplés du carbone et de l'azote dans la biosphère et le sol (Balesdent et al. 2005)

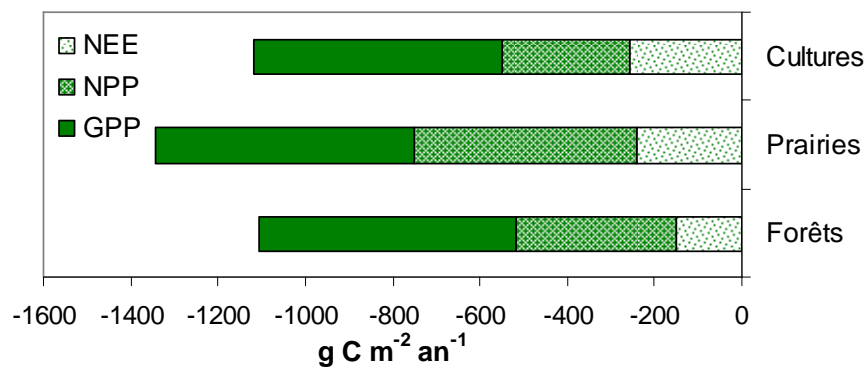
Le carbone de la matière organique transformé par l'activité microbienne peut suivre trois chemins, soit être rejeté dans l'atmosphère sous forme de  $\text{CO}_2$ , soit être assimilé et transformé

<sup>7</sup> Organismes autotrophes ayant la capacité de produire de la matière organique en procédant à la réduction de matière inorganique

<sup>8</sup> Qualifie un organisme qui assure sa subsistance en assimilant des substances organiques et incapable de produire ces substances organiques à partir de matière minérale

en biomasse microbienne, soit être incorporé et stocké dans les substances humiques du sol (Davet 1996).

La photosynthèse du couvert prairial reflète la production primaire brute de l'écosystème (GPP). Deux synthèses récentes sur 28 sites expérimentaux en Europe (Soussana *et al.* 2007, Schulze *et al.* 2009) ont montré que la GPP des prairies était comparable à celle des forêts européennes (Figure 8). Les entrées de carbone dans l'écosystème prairial se font par la production primaire nette (NPP<sup>9</sup>) de l'écosystème (Schlesinger 1991). Le bilan des échanges de CO<sub>2</sub> avec l'atmosphère (NEE<sup>10</sup> ou productivité nette de l'écosystème) atteint approximativement 242 gC par m<sup>2</sup> et par an pour les prairies en Europe (Soussana *et al.* 2007, Schulze *et al.* 2009) (Figure 8). La productivité primaire nette des prairies et le bilan des échanges de CO<sub>2</sub> avec l'atmosphère sont supérieurs à celui des forêts (Figure 8 ; Schulze *et al.* 2009).



**Figure 8 : Flux de CO<sub>2</sub> au sein de différents écosystèmes européens en relation avec leur productivité. (D'après Schulze *et al.* 2009)**

Le bilan des flux de GES à l'échelle systémique, et le stockage de carbone qui peut en résulter font l'originalité des écosystèmes prairiaux (Soussana *et al.* 2010). Afin de réaliser le bilan réel du carbone stocké par les prairies, il faut tenir compte de toutes les entrées et sorties incluant les modalités de gestion et d'exploitation comme l'apport d'engrais organique au sol (importation de C), les pratiques de fauches et / ou de pâture, le type de productions, bovine

<sup>9</sup> NPP : correspond au CO<sub>2</sub> accumulé par la plante par la voie de la photosynthèse moins le CO<sub>2</sub> utilisé par la plante pour la respiration (respiration autotrophes)

<sup>10</sup> NEE : correspond au CO<sub>2</sub> accumulé par la plante par la voie de la photosynthèse moins le CO<sub>2</sub> relâché par la respiration hétérotrophe des organismes telluriques et par la respiration autotrophe des plantes.

ou laitière (exportations de C), les pertes de carbone par le feu, par la production entérique de méthanes) par l'érosion du sol et par le lessivage (Figure 9). En tenant compte de tous ces flux, les prairies européennes s'avèrent malgré tout être d'importants puits de carbone, stockant entre 0.5 à 1 tonne de C par hectare et par an (Soussana et al. 2010).

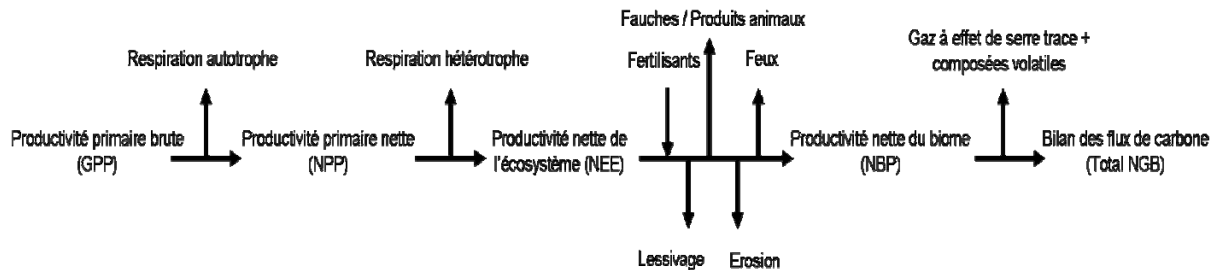


Figure 9 : Le bilan de carbone d'une prairie gérée correspond au bilan de plusieurs flux (D'après Schulze et al. 2009)

D'un point de vue agronomique, la production végétale en prairie permanente forme la base des ressources fourragères pour de nombreux systèmes d'élevage, par l'exploitation directe par des ruminants domestiques de la biomasse produite (*i.e.* par pâture), soit de manière indirecte (*i.e.* fauche et utilisation à l'étable des fourrages). La production des prairies permanentes varie selon la composition botanique de la flore et les modes de gestion appliqués au système (Pontes *et al.* 2007). Pour l'essentiel, la production des prairies permanentes de montagne s'effectue au printemps (70 à 80%, Arnaud *et al.* 1978), lors de la phase reproductrice des graminées (dominantes dans les prairies). Cette saison est la plus favorable à la croissance des herbacées: eau non limitante, température moyenne journalière proche de l'optimum physiologique des graminées (15°C), intensité lumineuse et photopériode favorables à l'activité photosynthétique et enfin minéralisation importante de l'azote dans le sol (Gillet 1980 ; Parsons *et al.* 1988). En général, la production végétale en prairie permanente fournit environ 49 millions de tonnes de matière sèche par an (*i.e.* ensilage ou foin) soit 65 % de la production de toutes les prairies en France (*i.e.* en incluant les prairies temporaires<sup>11</sup> et les prairies artificielles<sup>12</sup>, Agreste 2009). En Auvergne, la prairie permanente fournit environ 70 % des ressources alimentaires des herbivores domestiques (Agreste 2009).

<sup>11</sup> < 5ans, prairies semées en graminées ou graminées-légumineuses

<sup>12</sup> < 5ans, prairies semées en légumineuses pures, souvent fauchées

### 2.2.2 Les flux de GES traces

Les deux principaux gaz en prairie sont le méthane ( $\text{CH}_4$ ) et l'oxyde nitreux ( $\text{N}_2\text{O}$ ). La principale source de  $\text{CH}_4$  en prairie est la fermentation entérique des ruminants. Des quantités importantes de  $\text{CH}_4$  peuvent aussi être produites dans les sols où prévalent des conditions anoxiques, tels que les sols de rizières ou de zones inondées comme les marais (Roger *et al.* 1999). Le travail de ma thèse ne reposant pas sur un périmètre d'analyse incluant les animaux, ni sur un modèle d'étude présentant les conditions environnementales favorables à la production de  $\text{CH}_4$ , je centrerai cette partie bibliographique sur les flux de  $\text{N}_2\text{O}$ .

L'écosystème prairial peut aussi être une source et un puits d'azote, capable de le fixer (en présence de Fabacées) sous la forme de diazote atmosphérique ( $\text{N}_2$ ) ou bien de le réémettre soit sous cette même forme soit sous forme de  $\text{N}_2\text{O}$  (Figure 7). Le  $\text{N}_2\text{O}$  est un influant gaz à effet de serre de part son pouvoir de réchauffement global<sup>13</sup> (*i.e.* approximativement 300 fois supérieur au pouvoir de réchauffement du  $\text{CO}_2$ ; GIEC 2007) et le composant le plus important dans la déplétion de l'ozone<sup>14</sup> atmosphérique (Ravishankara *et al.* 2009). Les émissions de  $\text{N}_2\text{O}$  sont principalement dues à l'activité des micro-organismes (dénitrification, nitrification, ammonification, ou la plus rare nitrification dénitrifiante ; Figure 10), et parfois à la dénitrification chimique. Les substrats à l'origine de cette production sont les résidus organiques des plantes et des animaux, l'ammonium ( $\text{NH}_4^+$ ) et le nitrate ( $\text{NO}_3^-$ ), qui peuvent être apportés sous forme d'engrais. Les émissions de  $\text{N}_2\text{O}$  par réduction microbienne de  $\text{NO}_3^-$  en  $\text{NH}_4^+$  semblent être négligeables (Knowles 1982). La dénitrification et la nitrification sont les principaux processus biologiques impliqués et sont réalisés chacun par des groupes fonctionnels spécifiques de la microflore du sol (Robertson & Tiedje 1987 ; Bremner 1997; Wrage *et al.* 2004) (Figure 10). Concernant le diazote ( $\text{N}_2$ ) atmosphérique, il peut être intégré au sol après fixation par des microorganismes du sol (symbiotique ou libre) et est par la suite réduit en  $\text{NH}_4^+$ . Alternativement, des formes réactives d'azote ( $\text{NO}_3^-$  et  $\text{NH}_3^+$ ) peuvent être amenées au sol par déposition humide (pluies) ou déposition sèche.

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<sup>13</sup> Le pouvoir de réchauffement global d'un gaz se définit comme la puissance radiative que le gaz à effet de serre en question renvoie vers le sol cumulé sur une durée de 100 ans. Cette valeur se mesure relativement au  $\text{CO}_2$

<sup>14</sup> Destruction de l'ozone en haute atmosphère

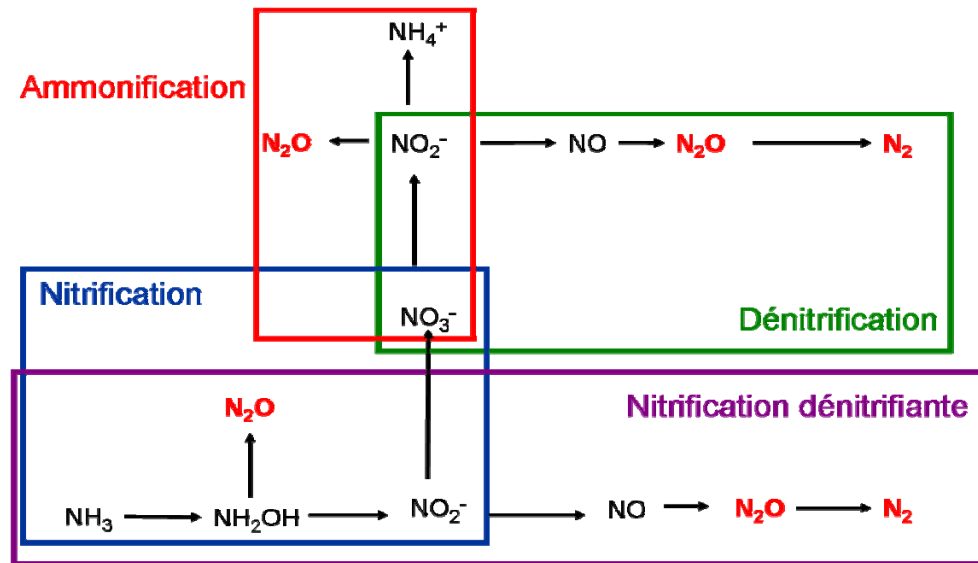


Figure 10 : Réactions de transformation de l'azote dans le sol (d'après Davet 1996)

Dans le sol, une quantité considérable de  $\text{NH}_4^+$  est utilisée par les plantes et les microorganismes (*i.e.* forte compétition pour le substrat) et la part restante est transformée en  $\text{NO}_3^-$  par des bactéries et des archaebactéries oxydant le  $\text{NH}_4^+$  par nitrification (Figure 7 et 10). Les ions  $\text{NO}_3^-$  quand ils ne sont pas absorbés par les plantes, sont quant à eux convertis *via* dénitrification en  $\text{N}_2$  ou autres formes intermédiaires  $\text{NO}$  et  $\text{N}_2\text{O}$  libérés dans l'atmosphère. Les processus de dénitrification sont effectués par un ensemble de bactéries possédant les gènes de fonctions pour la dénitrification (Figure 11). Ainsi la transformation des ions  $\text{NO}_3^-$  en ions  $\text{NO}_2^-$  se fait par l'intermédiaire des enzymes nitrate réductase, celle des ions  $\text{NO}_2^-$  en  $\text{NO}$  *via* la famille d'enzymes nitrite réductase. Le  $\text{NO}$  formé peut ensuite être libéré dans l'atmosphère ou être utilisé par les bactéries possédant les enzymes *Nor* conduisant à la formation de  $\text{N}_2\text{O}$ . Si l'oxydation du nitrate est totale, le  $\text{N}_2\text{O}$  sera transformé en  $\text{N}_2$  grâce aux enzymes *Nos*. Si les ions  $\text{NO}_3^-$  ne sont pas tous utilisés par les plantes ou les microorganismes, ils peuvent alors être lixiviés vers les couches profondes du sol voire les nappes phréatiques.

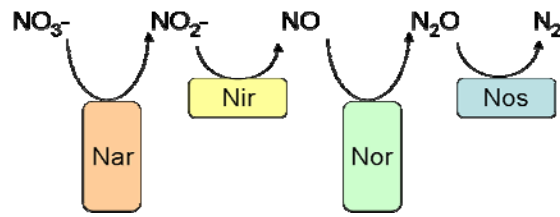


Figure 11 : Dénitrification et implication des différentes enzymes réductrices. Nar, nitrate réductase ; Nir, nitrite réductase ; Nor, oxyde nitrique réductase ; Nos, oxyde nitreux reductase (d'après Wallenstein et al. 2006)

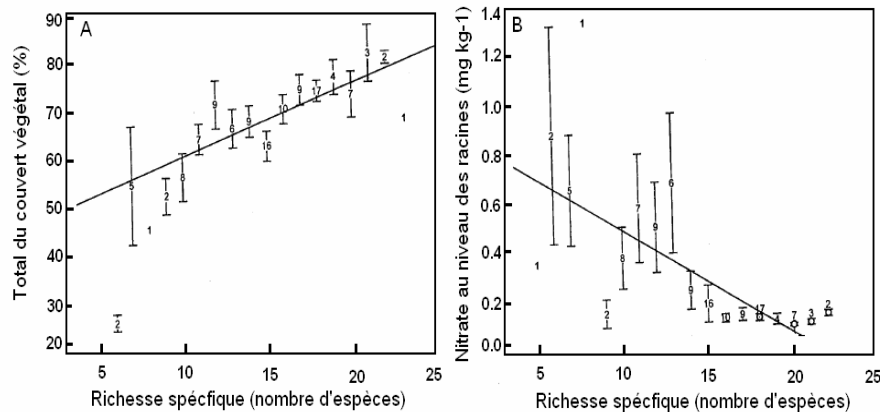
### 2.2.3 La diversité végétale

Les changements de diversité<sup>15</sup> sont connus pour être complexes et pour répondre aux changements induits par l'Homme (Vitousek 1994 ; Pimm *et al.* 1995). L'ampleur de ces changements de diversité est si fortement liée aux processus des écosystèmes (Chapin *et al.* 1997 ; Tilman *et al.* 1997) et à l'utilisation sociétale des ressources (Costanza *et al.* 1997) que les changements de diversité sont maintenant considérés comme faisant partie du changement global (Walker & Steffen 1996, Sala *et al.* 2000).

De nombreuses études indiquent que les changements de composition et de diversité des communautés végétales affectent la production primaire des prairies (Tilman & Downing 1994 ; Hooper *et al.* 2005 ; Fischer *et al.* 2008) ainsi que la fertilité du sol (Tilman *et al.* 1996). Une richesse spécifique élevée procure à l'écosystème une capacité d'adaptation vis-à-vis des variations de l'environnement physique et biologique et par conséquent une certaine stabilité de son fonctionnement (Figure 12). Cet effet, dit d'«assurance biologique» (Yachi & Loreau 1999) repose sur un constat simple : toutes les espèces ne répondent pas de la même façon aux fluctuations de l'environnement, ce qui implique que les conditions de compétition entre espèces sont modifiées et que certaines espèces accèdent au statut de dominantes et d'autres à celui de dominées. La diversité végétale est donc un facteur important de contrôle de la production primaire (Loreau *et al.* 2001 ; Hooper *et al.* 2005 ; Cardinale *et al.* 2007 ; Gross *et al.* 2009), et le changement climatique est l'un des facteurs les plus importants qui va jouer sur la diversité des prairies (Sala *et al.* 2000).

<sup>15</sup> Les changements de diversité au niveau des écosystèmes sont définis comme des changements dans le nombre et l'abondance relative des espèces qui ont lieu naturellement dans l'écosystème





**Figure 12 :** Relations entre (A) la richesse spécifique et la proportion de couvert végétal en prairies naturelles, et (B) entre la richesse spécifique et l'utilisation de l'azote dans le sol (d'après Tilman et al. 1996)

Le lien entre la diversité et le fonctionnement de l'écosystème peut être déterminé par la composition des espèces présentes (Givnish 1994 ; Chapin *et al.* 1995), ou leur nombre (*i.e.* richesse spécifique, Tilman 1996), mais aussi par des caractéristiques clés propres aux espèces et aux écosystèmes étudiés (Diaz & Cabido 2001 ; Lavorel & Garnier 2002) et par le nombre de groupes fonctionnels<sup>16</sup> de l'écosystème (Hooper & Vitousek 1997). L'approche fonctionnelle est basée sur l'hypothèse que certaines caractéristiques des individus (ou traits de réponse) jouent un rôle clé dans la réponse des populations et des communautés aux changements du milieu (traits de réponses) et ont des effets sur le fonctionnement de l'écosystème (traits d'effet) (Diaz & Cabido 2001 ; Lavorel & Garnier 2002) (Figure 13). Cette approche s'est révélée pertinente pour rendre compte de la réponse de la végétation à différentes perturbations comme le pâturage (Louault *et al.* 2005 ; Pontes *et al.* 2007), ou encore le feu (Starfield & Chapin 1996), ainsi que pour comprendre les effets de la composition fonctionnelle sur les propriétés des écosystèmes (production, résilience/stabilité, Diaz & Cabido 2001). A ce jour, cette approche reste encore peu utilisée pour étudier les réponses des communautés au changement climatique.

<sup>16</sup> La plupart des groupes fonctionnels dans les manipulations sur la biodiversité considèrent les formes de vie comme base de classification (dicotylédones, graminées, légumineuses...) (Raunkiaer 1934)

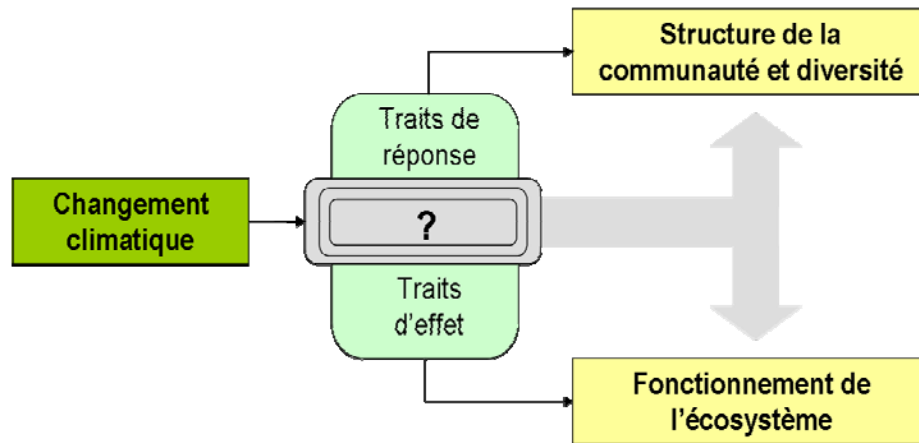


Figure 13 : Impacts du changement climatique sur la structure des communautés et le fonctionnement de l'écosystème. (D'après Lavorel & Garnier 2002)

Depuis une dizaine d'années, deux hypothèses concurrentes tentent d'expliquer comment la diversité d'une communauté peut influencer les processus de l'écosystème, l'hypothèse de diversité ou 'the diversity hypothesis' (Tilman 1997) et l'hypothèse des espèces dominantes ou 'the mass ratio hypothesis' (Grime 1998). 'The diversity hypothesis' propose que la diversité des organismes et de leurs attributs fonctionnels influencent des processus de l'écosystème : plus un écosystème est riche (diversité spécifique) mieux il se maintient face aux perturbations environnementales (Tilman 1997). 'The mass ratio hypothesis' propose au contraire que les processus de l'écosystème sont déterminés par les traits fonctionnels des espèces dominantes dans la communauté et sont relativement insensibles à la richesse spécifique (Grime 1998). Ces deux hypothèses ne sont pas mutuellement exclusives. Il est même probable qu'elles soient complémentaires, reflétant deux voies possibles de la régulation des processus de l'écosystème (Thompson *et al.* 2005). Par exemple, dans une étude expérimentale en mésocosme, Klumpp et Soussana (2009) ont montré que certains processus étaient particulièrement affectés par les traits des espèces dominantes (stockage de carbone dans le sol), alors que d'autres étaient surtout affectés par la diversité de ces traits (productivité primaire aérienne).

### 2.3 Impacts des changements climatiques sur la structure et le fonctionnement de la prairie

Le changement climatique affecte directement la production, les espèces végétales et

microbiennes ou encore les processus de fonctionnement de l'écosystème (*i.e.* photosynthèse) en modifiant leur environnement physique et affecte indirectement ces mêmes processus en jouant sur les interactions interspécifiques comme la prédation et la compétition ou encore en modifiant les valeurs des traits fonctionnels (Figure 14). Ces effets indirects peuvent amplifier ou neutraliser les effets directs du changement climatique. Adler (2009) suggère que les effets directs négatifs du réchauffement sur une espèce de plante peuvent être compensés par exemple en réduisant l'abondance des ennemis de cette espèce. Les effets indirects obtenus par médiation des interactions interspécifiques peuvent donc modifier l'impact des effets directs de changement climatique (Figure 14).

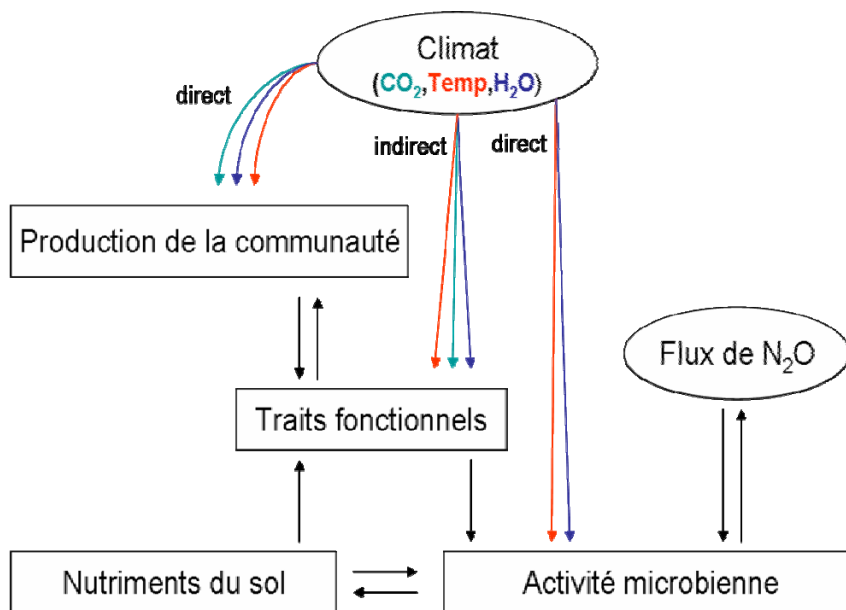


Figure 14 : Impact du changement climatique en prairie, effets directs et indirects (Adapté de Lavorel & Garnier 2002)

### 2.3.1 Impact d'une élévation en CO<sub>2</sub> atmosphérique sur le fonctionnement des prairies

#### *Echanges gazeux plantes-atmosphère et production végétale sous CO<sub>2</sub> atmosphérique élevé*

L'impact d'une augmentation des teneurs en CO<sub>2</sub> atmosphérique sur les plantes et plus particulièrement les feuilles a fait l'objet de plus de 4000 publications (Körner 2000). En prairies, tout comme dans d'autres écosystèmes terrestres, une augmentation du taux de photosynthèse a été démontrée (Körner 1995 ; Paterson *et al.* 1997) sous des teneurs élevées en CO<sub>2</sub> atmosphérique. Des changements dans la biochimie des plantes sont souvent

rapportés, le CO<sub>2</sub> ayant des effets sur l'activité carboxylase<sup>17</sup> des plantes (incluant la Rubisco) se traduisant par une augmentation de l'assimilation nette des feuilles et des concentrations de sucres et autres carbohydrates non-structuraux dans les plantes (Cotrufo *et al.* 1994). Bien que les réponses stomatiques diffèrent beaucoup entre espèces, en moyenne la conductance stomatique en prairie sous CO<sub>2</sub> élevé baisse fortement, soit de 30 à 50% (Ainsworth *et al.* 2003) et la transpiration est réduite de 5 à 10 % (Stocker *et al.* 1997). Afin de maintenir un équilibre dans les ressources (*i.e.* azote et autres nutriments) allouées aux réactions qui contrôlent la photosynthèse, les plantes s'acclimatent aux teneurs élevées en CO<sub>2</sub> atmosphérique (Sage 1989; Gunderson & Wullschleger 1994; Drake *et al.* 1997). L'acclimatation de la photosynthèse est souvent attribuée à une diminution de la vitesse de carboxylation maximale (V<sub>cmax</sub>) et à un investissement plus fort dans la Rubisco<sup>18</sup> (Rogers & Humphries 2000). De plus, l'acclimatation photosynthétique est fréquemment reliée à une diminution de la concentration azotée au niveau de la feuille et de la plante entière (Nowak *et al.* 2004).

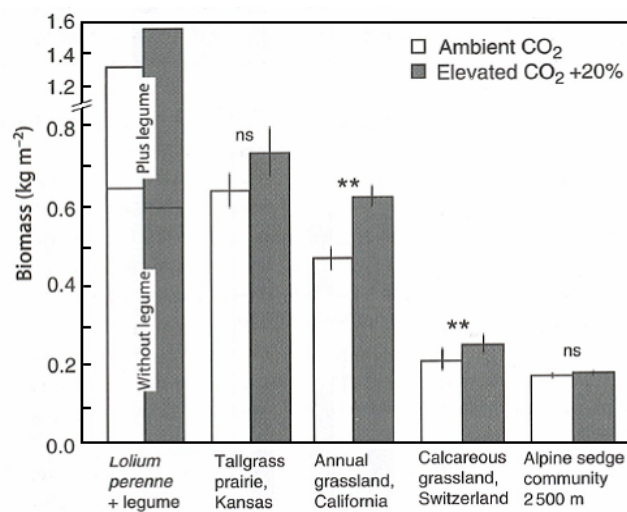
Malgré l'impact positif du CO<sub>2</sub> à l'échelle foliaire, la production aérienne sous CO<sub>2</sub> élevé n'est pas aussi importante que celle prédite en étudiant la seule voie photosynthétique (Poorter 1993) et on constate une variation importante de cette production entre les différentes études (Figure 15). Les facteurs susceptibles d'expliquer ces erreurs de prédiction sont l'absence de prise en compte d'une limitation de la disponibilité en nutriments du sol (Niklaus 1998 ; Stitt & Krapp 1999 ; Morgan *et al.* 2001), d'une augmentation de l'allocation du carbone aux racines (Soussana *et al.* 1996) qui peuvent varier selon les espèces végétales (Poorter 1993) ou encore des changements dans les relations hydriques plantes-sol (Morgan *et al.* 2004). Les résultats de Morgan *et al.* (2004) suggèrent qu'une augmentation en CO<sub>2</sub> atmosphérique induit des rétroactions sur la teneur en eau du sol qui peuvent confondre les effets directs sur la production. La part relative de ces différents mécanismes reste encore à quantifier.

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<sup>17</sup> La carboxylase est une enzyme qui assure l'incorporation du CO<sub>2</sub> dans les molécules intermédiaires pour la synthèse de glucides.

<sup>18</sup> ribulose-1,5-diphosphate carboxylase/oxygénase est l'enzyme clé permettant la fixation du CO<sub>2</sub> dans la biomasse végétale en initiant le cycle de Calvin, en relation avec l'énergie solaire captée par la chlorophylle à l'origine des processus de photosynthèse.

L'exposition prolongée des communautés végétales à un enrichissement en CO<sub>2</sub> peut entraîner des modifications de la structure des communautés végétales (Leadley *et al.* 1999; Daepf *et al.* 2001; Teyssonneyre *et al.* 2002; Polley *et al.* 2003; Dukes *et al.* 2005; Kamman *et al.* 2005). Pourtant, il existe des temps de latence dans les réponses de la végétation (*i.e.* croissance, mortalité des plantes) à cet enrichissement en CO<sub>2</sub>, compliquant la prédiction des réponses futures de la biomasse aérienne aux changements environnementaux (Shaver *et al.* 2000; Dunne *et al.* 2004 ; Winkler & Herbst 2004; Kammann *et al.* 2005). Ces temps de latence, leur importance et leur rôle dans les réponses des écosystèmes prairiaux exposés au changement climatique restent encore mal connus et peu expliqués (Dukes *et al.* 2005).



**Figure 15 :** Comparaison des réponses de la biomasse à une élévation de CO<sub>2</sub> pour 4 écosystèmes naturels de prairies (pas de fertilisation) et un mélange artificiel légumineuses / *Lolium perenne*. Les étoiles indiquent des différences significatives et « ns » signifie non-significatif (d'après Körner *et al.* 2007)

#### Flux de N<sub>2</sub>O sous CO<sub>2</sub> atmosphérique élevé

Une élévation en CO<sub>2</sub> peut fortement affecter les transformations de l'azote du sol et les flux de N<sub>2</sub>O suite à une disponibilité accrue du carbone labile (facilement dégradable) pour les communautés microbiennes du sol et à une réduction de l'utilisation de l'eau par les plantes (Luo & Money 1999). Les études menées sur les flux de N<sub>2</sub>O et les activités microbiennes en prairies ont montré des réponses contradictoires, souvent liées au mode de gestion (fertilisation azotée ou non) ou aux conditions abiotiques (teneur en eau du sol). Ainsi, Ineson *et al.* (1997) ont montré une augmentation de 27% des émissions de N<sub>2</sub>O sous CO<sub>2</sub> élevé pour une culture pure de *Lolium perenne*. Baggs *et al.* (2003) ont réutilisé cette

expérience pour analyser les activités microbiennes potentielles (dénitrification et nitrification). La dénitrification augmente sous CO<sub>2</sub> élevé et devient la source principale des flux de N<sub>2</sub>O au champ, l'apport supplémentaire de carbone au sol fournissant une source d'énergie supplémentaire favorisant la dénitrification. Ces résultats ont également été observés en prairies plurispécifiques.(Arnone *et al.* 1998 ; Kettunen *et al.* 2007 ; Kamman *et al.* 2008). Néanmoins, certains auteurs n'ont pas trouvé d'impact du CO<sub>2</sub> sur les flux de N<sub>2</sub>O (Billings *et al.* 2002 ; Barnard *et al.* 2004), mettant même en avant des effets négatifs (diminution des flux de N<sub>2</sub>O pour Kettunen *et al.* 2005). Les mécanismes complexes à l'origine de cette variabilité restent encore mal identifiés et mal compris.

#### *Diversité végétale sous CO<sub>2</sub> atmosphérique élevé*

En général, les plantes en C<sub>3</sub> répondent plus que les plantes en C<sub>4</sub> à une augmentation du CO<sub>2</sub> (Paterson *et al.* 1997). Les plantes fixatrices d'azote, quant à elles, sont plus réceptives que les non-fixatrices à une augmentation du CO<sub>2</sub> du fait de leur autonomie pour les ressources en azote (Nowak *et al.* 2004). Plusieurs résultats indiquent que la disponibilité en eau est un facteur clé dans les réponses spécifiques des espèces à une élévation de CO<sub>2</sub> (Owensby *et al.* 1993 ; Morgan *et al.* 2004), bien que cette hypothèse ne soient pas validée par toutes les études (Zavaleta *et al.* 2003 ; Nowak *et al.* 2004). Les changements dans la contribution relative des espèces dans une communauté prairiale sous CO<sub>2</sub> élevé peuvent altérer sa production et sa diversité en termes de richesse spécifique et même d'équitabilité (Potvin & Vasseur 1997 ; Chapin *et al.* 2000; Körner 2001). La plupart des études ont montré une augmentation de la contribution relative des dicotylédones (*i.e.* légumineuses) et une diminution de celle des monocotylédones (*i.e.* graminées) (Owensby *et al.* 1993 ; Potvin & Vasseur 1997 ; Teyssonneyre *et al.* 2002 ; Niklaus & Körner 2004) à condition dans certains cas que le phosphore ne soit pas limitant (Stöcklin *et al.* 1998 ; Soussana & Lüscher 2007).

### **2.3.2 Impact d'un réchauffement sur le fonctionnement et la structure des prairies**

Etant donné l'importance de la température dans la régulation des principaux processus chimiques et biologiques, le réchauffement climatique est attendu pour avoir des effets significatifs sur la structure et le fonctionnement des écosystèmes (Rustad & Norby 2002). La température est un facteur régulant la photosynthèse, la respiration, la minéralisation de

l'azote, la nitrification et la dénitrification, ou encore la production végétale (DeBoeck *et al.* 2008), et ce d'autant plus sous des climats tempérés (Rustad *et al.* 2001).

### *Impact du réchauffement sur la production végétale et la photosynthèse*

Chaque processus enzymatique a une température optimale. L'augmentation des températures de surface est connue pour activer la vitesse des processus enzymatiques et en particulier celui de la respiration, mais aussi ceux régissant la croissance des organismes végétaux (Taiz & Zeiger, 2010).

La croissance de la végétation prairiale est fortement dépendante de la température en fin de période hivernale et au printemps, c'est-à-dire pendant la croissance végétative (production de feuilles). Lors de l'entrée de la plante dans la croissance reproductive (formation des épis), la longueur du jour devient le facteur primordial. Pendant cette phase reproductive, il y a une accélération de la vitesse de croissance (Lemaire & denoix, 1987) s'accompagnant d'une augmentation de la capacité photosynthétique des feuilles pour satisfaire les demandes en assimilats nécessaires à la réalisation de l'épi. Ce stade phénotypique dépassé, les températures ne sont plus un facteur limitant pour la production de biomasse aérienne. Cependant de fortes chaleurs en été limitent la croissance des espèces prairiales. Les optimums de croissance sont propres à chacune des espèces (*i.e.* 20-22°C pour le ray-grass anglais, 25°C pour le dactyle et la fétuque, Soussana & Lemaire, 2007)

Contrairement aux résultats attendus, les études en prairie montrant clairement une augmentation de la production végétale en réponse à une élévation de température sont relativement rares (Rustad *et al.* 2001 ; Dukes *et al.* 2005 ; Gielen *et al.* 2007 ; DeBoeck *et al.* 2008 ; Grime *et al.* 2008). Ces études montrent qu'il y a une différence entre l'impact auquel les scientifiques s'attendent et la réponse réelle des écosystèmes en conditions naturelles. L'absence d'effets stimulants du réchauffement sur la production en période de croissance peut s'expliquer par des contraintes hydriques et nutritives, c'est-à-dire une diminution de l'humidité du sol et de la disponibilité des nutriments suite à l'évapotranspiration accrue (Rustad *et al.* 2001). En général, les réponses de la production au réchauffement sont plus prononcées dans des écosystèmes froids (*i.e.* steppes, Rustad *et al.* 2001). Sous ces climats plus hostiles, l'augmentation de la production végétale induite par le réchauffement s'explique

soit par une augmentation de la photosynthèse, soit par une prolongation des périodes de croissance au cours de la saison (Fitter *et al.* 1997; Dunne *et al.* 2003; Cleland *et al.* 2006; Hovenden *et al.* 2008), ou par une mise à disposition des ressources nutritive accrue (Nord & Lynch 2009), résultant de l'augmentation de la décomposition et de la minéralisation des litières.

#### *Impact du réchauffement sur les flux de N<sub>2</sub>O*

L'impact de la température sur les processus microbiens (nitrification et dénitrification) à l'origine des flux de N<sub>2</sub>O a bien été étudié dans la littérature (de Klein & van Lotjstijn 1996 ; Clayton *et al.* 1997; Smith *et al.* 1998; Gödde & Conrad 1999; Bateman & Baggs 2005; Jones *et al.* 2005; Hyde *et al.* 2006; Flécharde *et al.* 2007; Kammann *et al.* 2008). Keeney (1979) a observé qu'au dessus de 15°C la dénitrification augmente significativement avec la température du sol. Dans le même sens, Glatzel *et al.* (2006) montrent que de hautes températures stimulent fortement l'activité microbienne quelle qu'elle soit à l'origine des flux de N<sub>2</sub>O. Néanmoins les réponses au réchauffement de la nitrification et de la dénitrification en conditions naturelles sont variables en fonction notamment des teneurs en eau et en azote minéral du sol (Shaw & Harte 2001; Emmett *et al.* 2004; Horz *et al.* 2004; Hart 2006; Malchair *et al.* 2010; Szukics *et al.* 2010). De ce fait, les flux de N<sub>2</sub>O présentent une grande variabilité temporelle en réponses aux facteurs abiotiques (température et teneur en eau du sol, Flécharde *et al.* 2007), qui peuvent interférer avec l'impact du réchauffement sur les flux de N<sub>2</sub>O au champ (Billings *et al.* 2002; Kettunen *et al.* 2005; Flécharde *et al.* 2007).

#### *Impact du réchauffement sur la diversité*

Les études disponibles n'ont montré que peu de changements de la composition botanique sous l'effet du réchauffement (Price & Waser 2000 ; Grime *et al.* 2008). Comme observé sous élévation en CO<sub>2</sub> atmosphérique le groupe fonctionnel des dicotylédones semble être plus affecté que ceux des graminées et des légumineuses. DeValpine & Harte (2001) ont rapporté des réponses différentes au réchauffement suivant le type de dicotylédones étudiées : sur 11 espèces 2 ont répondu positivement (leur abondance relative augmentant) et 6 négativement, (leur abondance relative diminuant). Zavaleta *et al.* (2003) suggèrent que le réchauffement a une tendance (non significative) à augmenter la diversité spécifique des prairies, favorisant



l'abondance des dicotylédones à condition que les graminées ne soient pas dominantes. Les plantes en C<sub>4</sub> pour leur part semble être elles aussi favorisées sous réchauffement climatique davantage que celle en C<sub>3</sub>, ce qui peut s'expliquer par l'optimum thermique plus élevé chez les plantes en C<sub>4</sub> pour la photosynthèse et la croissance aérienne (Sherry *et al.* 2008). A ce jour, l'évolution des traits fonctionnels végétaux en réponse au réchauffement n'a pas encore fait l'objet d'études *in situ*, bien qu'elle soit fortement impliquée dans la réponse globale du fonctionnement et de la structuration de l'écosystème prairial.

### **2.3.3 Impact de la sécheresse en prairie**

#### *Impact de la sécheresse sur la production végétale et sur la photosynthèse*

Les conséquences de déficits hydriques sur la photosynthèse et la morphogénèse d'une plante sont bien connues (voir Figure 16). Elles concernent aussi bien l'allocation des assimilats entre compartiments aériens et souterrains que la croissance aérienne, la régulation stomatique et la morphogénèse. La sécheresse ou la variation des précipitations, tant dans leur intensité que dans leur fréquence, affectent non seulement la photosynthèse et la production végétale (Sala *et al.* 1988 ; Huxman *et al.* 2004), mais aussi la distribution spatiale des espèces (Yin *et al.* 2005), les taux de germination et de survie des plantules (Lloret *et al.* 2004 ; Padilla & Pugnaire 2006) ainsi que le fonctionnement des communautés microbiennes (Avrahami *et al.* 2009). La diminution de la photosynthèse a des conséquences sur le métabolisme du carbone, l'activité de certaines enzymes impliquées dans la circulation des assimilats étant régulée en réponse à un déficit hydrique (Zinselmeyer *et al.* 1999). La sécheresse et les changements des patrons régionaux de précipitations peuvent avoir des effets immédiats sur la dynamique des écosystèmes et peuvent causer des variations dans la production de biomasse aérienne pendant et après la perturbation ou à plus long terme selon leur capacité de résilience (Seastedt & Knapp 1993; Blair 1997; Knapp & Smith 2001). Après une sécheresse importante, le retour d'un écosystème prairial à des niveaux de productivité comparables à ceux mesurés avant une perturbation peut aller jusqu'à deux ans (Polis *et al.* 1997; Herbert *et al.* 1999). Les sécheresses sévères ont souvent des effets négatifs sur la production végétale à court terme (Tilman & Downing 1994; Briggs *et al.* 1995) mais aussi à long terme (Lauenroth & Sala 1992). Un exemple récent de l'impact d'une forte sécheresse sur les écosystèmes concerne la canicule et la sécheresse de l'été 2003 en Europe. La productivité des

écosystèmes a été réduite de près de 30 % (Ciais *et al.* 2005) s'accompagnant d'une forte réduction de leur activité de puits de carbone dans la plupart des sites européens étudiés.

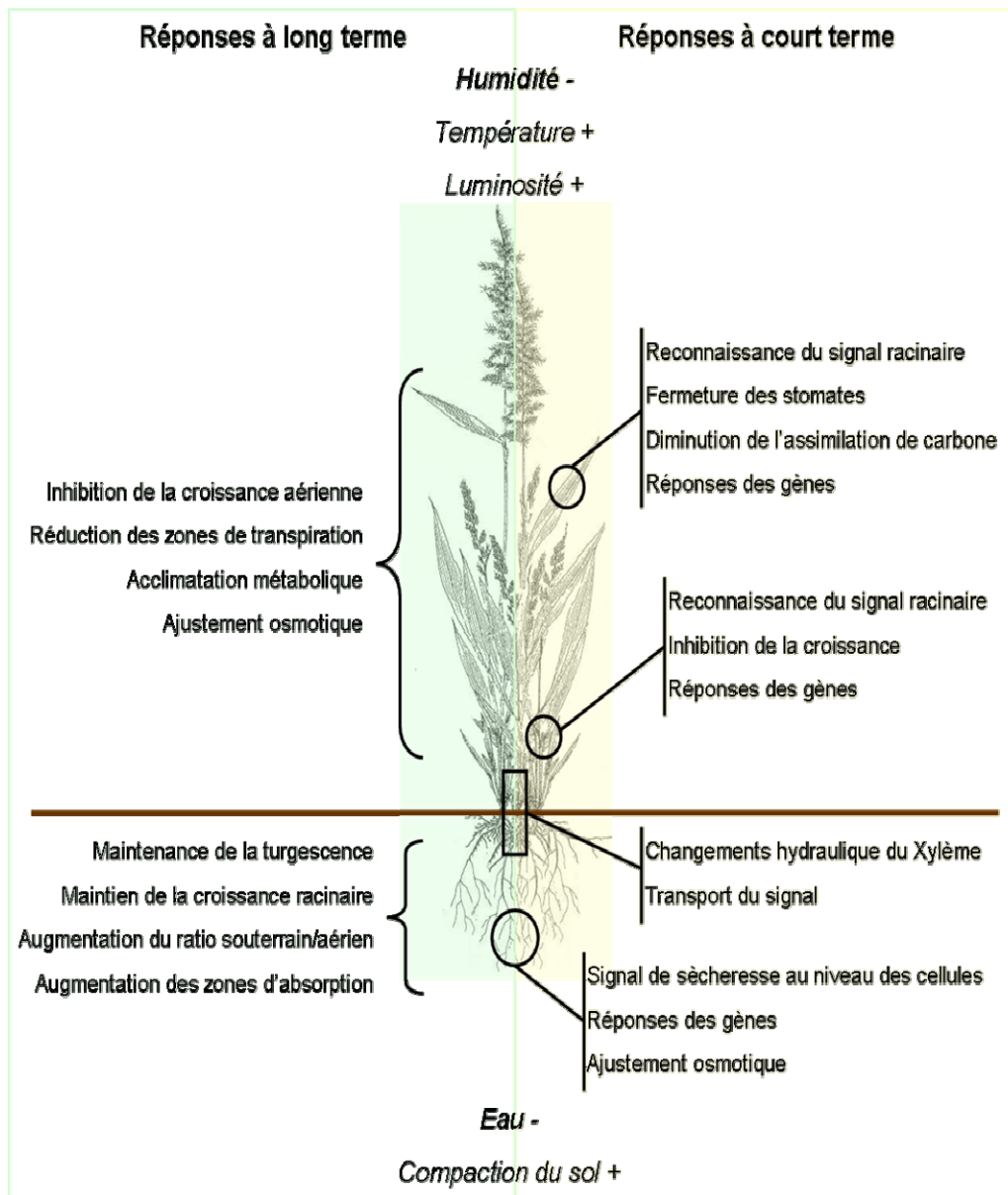


Figure 16 : Réponse de la plante à une sécheresse. A gauche les réponses à long terme et l'acclimatation possible ; à droite les réponses à court-terme. (Adapté de Chaves *et al.* 2003).

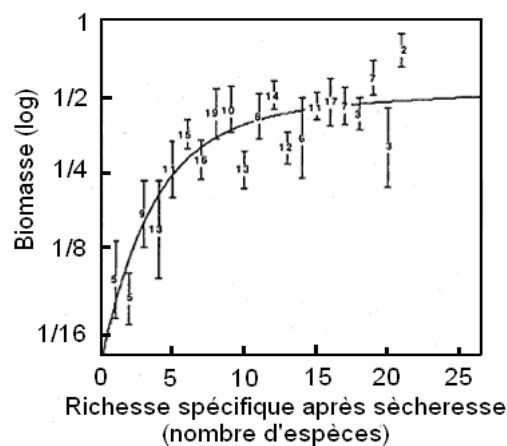
#### Impact d'une sécheresse sur les flux de $N_2O$

Lors d'une sécheresse, la diminution de la teneur en eau du sol affecte les flux de  $N_2O$  (Del Prado *et al.* 2006; Flécharde *et al.* 2007), généralement en les réduisant (Dobbie & Smith 2001; Smith *et al.* 2003). L'impact d'une réduction de la teneur en eau du sol sur les processus

microbiens du sol est bien documenté (Rustad 2001, Barnard *et al.* 2005; Bateman & Baggs 2005). La dénitrification est connue pour être principalement contrôlée par le degré d'anaérobiose du sol directement relié au niveau de saturation en eau (Linn & Doran 1984). De plus, la teneur en eau du sol détermine la balance entre nitrification et dénitrification *via* les changements d'aération du sol et de teneur en oxygène (Smith *et al.* 2003).

#### *Impact d'une sécheresse sur la diversité végétale*

Tilman & Downing (1994) ont montré pour les prairies, qu'une forte richesse spécifique permettait aux communautés végétales de mieux résister aux événements de sécheresse (Figure 17). Cette relation entre la richesse spécifique et la résistance de l'écosystème à la sécheresse (également indiquée par Pfisterer & Schmid 2002 ; Grime *et al.* 2008) ne semble cependant pas être constante dans la littérature (Wang *et al.* 2007). Ces derniers suggèrent que la réduction de biomasse induite par une sécheresse serait sensible à l'état initial de la biomasse de la communauté végétale sans se soucier de la relation diversité-stabilité.



**Figure 17 : Relation entre la richesse spécifique et la production de biomasse aérienne (échelle logarithmique). (D'après Tilmann & Downing 1994)**

L'impact de la sécheresse sur la diversité peut aussi être lié à l'intensité de la sécheresse. Ainsi une réduction de 50 % des précipitations sur une prairie semi-aride (Miranda *et al.* 2009) provoque une perte d'espèces pour la famille des graminées annuelles, alors qu'une réduction de 25% n'affecte pas cette diversité. Ceci illustre l'existence d'un effet de seuil qui traduit une limite à l'adaptation des espèces. Concernant les prairies plus humides

(Fay *et al.* 2000 ; Knapp *et al.* 2002). Les évènements de sécheresse affectent différemment les espèces dominantes et dominées. Les espèces dominantes possèdent souvent une tolérance plus forte à la sécheresse, qui pourrait s'expliquer par une plus grande plasticité génétique (Fay *et al.* 2003).

### **2.3.4 L'apport des facteurs multiples dans la compréhension du changement climatique**

Peu d'expériences à l'échelle des écosystèmes prairiaux ont examiné l'impact de plus de deux facteurs climatiques en interaction. La seule expérience multifactorielle de grande envergure en prairie menée à ce jour est le dispositif "Jasper Ridge Global Change Experiment" débuté en 1998 en Californie. Ce dispositif a pour objectif de tester les effets combinés ou pas d'une augmentation des températures (+1 °C), de la teneur en CO<sub>2</sub> atmosphérique (+ 300 ppm), des précipitations (+ 50 %) et de la déposition d'azote (+ 7 gNm<sup>-2</sup>an<sup>-1</sup>) sur des prairies permanentes en climat Méditerranéen (hiver froid et pluvieux et été très sec). Après cinq ans, les résultats de ce projet indiquent des effets de chaque facteur, mais suggèrent que les interactions entre les facteurs du changement climatique sont rares et ont des influences limitées sur la production de biomasse à long terme (Dukes *et al.* 2005). Certaines réponses de la production aux combinaisons climatiques apparaissent additive (réchauffement et déposition d'azote), alors que d'autres sont intermédiaires entre les facteurs simples (réchauffement et précipitations) (Shaw *et al.* 2002). Il peut y avoir aussi des interactions transitoires entre facteurs (CO<sub>2</sub> et précipitations) qui peuvent se confondre avec la variabilité météorologique et/ou la disponibilité des nutriments du sol (Morgan *et al.* 2004), ce qui souligne l'importance d'études à long terme. A ce jour aucun travail n'a étudié l'impact d'un changement climatique multifactoriel sur les traits fonctionnels et sur la diversité fonctionnelle. Beaucoup de questions restent donc en suspens quant aux conséquences fonctionnelles d'une évolution de la structure de la communauté sous changement climatique.

Les flux de N<sub>2</sub>O ont eux aussi été très peu étudiés en conditions expérimentales de changement climatique et encore moins dans des expériences multifactorielles, que ce soit au niveau microbien avec des mesures d'activités potentielles, comme à l'échelle de l'écosystème, avec des mesures de flux de N<sub>2</sub>O au champ. Seuls Barnard *et al.* 2006, sur le dispositif Jasper Ridge, se sont attachés à regarder les communautés microbiennes du point de vue des activités microbiennes. Les résultats de cette étude ont montré que la nitrification

diminuait sous la combinaison du CO<sub>2</sub> et de la déposition d'azote élevés, alors qu'elle augmentait dans le traitement déposition d'azote seul et diminuait dans le traitement CO<sub>2</sub> élevé seul. Ces résultats suggèrent une suppression de l'effet positif de l'apport sous CO<sub>2</sub> élevé. A l'inverse la dénitrification semble positivement affectée par la combinaison CO<sub>2</sub> - déposition d'azote. Les questions sont encore nombreuses sur les évolutions potentielles des flux de GES traces et des communautés microbiennes associées à leur production en prairies sous changement climatique. Etant donné les effets combinés connus de la température et la teneur en eau du sol sur la production de N<sub>2</sub>O (Flécharde *et al.* 2007), il est d'autant plus important d'étudier les flux de N<sub>2</sub>O dans des scénarios complexes.

### 3- Conclusions

Les expériences de forçage des écosystèmes par manipulation du micro-climat et de la teneur en gaz traces de l'atmosphère apparaissent encore aujourd'hui comme incontournables pour mieux comprendre et prédire les effets du changement climatique. Si par le passé les recherches centraient leur étude sur un seul facteur (*i.e.* enrichissement en CO<sub>2</sub>, sécheresse ou réchauffement) conduisant finalement à des résultats non représentatifs de la complexité de la réalité, il est désormais nécessaire de comprendre comment les différentes composantes du changement climatiques agissent sur le fonctionnement des prairies en ayant recours à des expérimentations simulant sur un scénario de changement climatique multifactoriel d'une ampleur comparable à celui attendu pour la fin du siècle.

### 4- Objectifs de la thèse et annonce du plan

Ce travail de thèse propose de contribuer à accroître notre compréhension de l'impact des facteurs climatiques futurs (seuls ou combinés) sur le fonctionnement d'un écosystème porteur d'enjeux sociétaux et écologiques forts. La question centrale de cette thèse est plus précisément **d'évaluer les impacts d'un changement climatique sur le fonctionnement de l'écosystème prairial en interaction avec sa biodiversité (végétale et microbienne) et plus particulièrement sur les flux de gaz à effets de serre.**

Les objectifs essentiels de ce travail sont : **(1)** de déterminer l'importance des facteurs climatiques sur le fonctionnement et la structure d'un écosystème prairial et **(2)** d'identifier

les processus et les mécanismes régissant ces modifications.

Ce travail de thèse s'est largement inspiré de la volonté nouvelle de coupler les processus et les mécanismes à l'échelle sol-plantes-atmosphère. Il s'est construit autour des processus détaillés dans l'état de l'art, c'est à dire la production de biomasse, les flux de CO<sub>2</sub> et de N<sub>2</sub>O et la diversité qu'elle soit végétale ou microbienne. Trois grandes parties ont été considérées. La première cible le fonctionnement de l'écosystème prairial sous changement climatique en termes de production de biomasse aérienne et de diversité. La seconde s'attache à étudier les échanges gazeux de CO<sub>2</sub> et leur régulation à l'interface entre végétation et atmosphère. La troisième cible les échanges gazeux de N<sub>2</sub>O dans le continuum sol-plante-atmosphère. Le cadre conceptuel de la thèse est précisé dans la Figure 18.

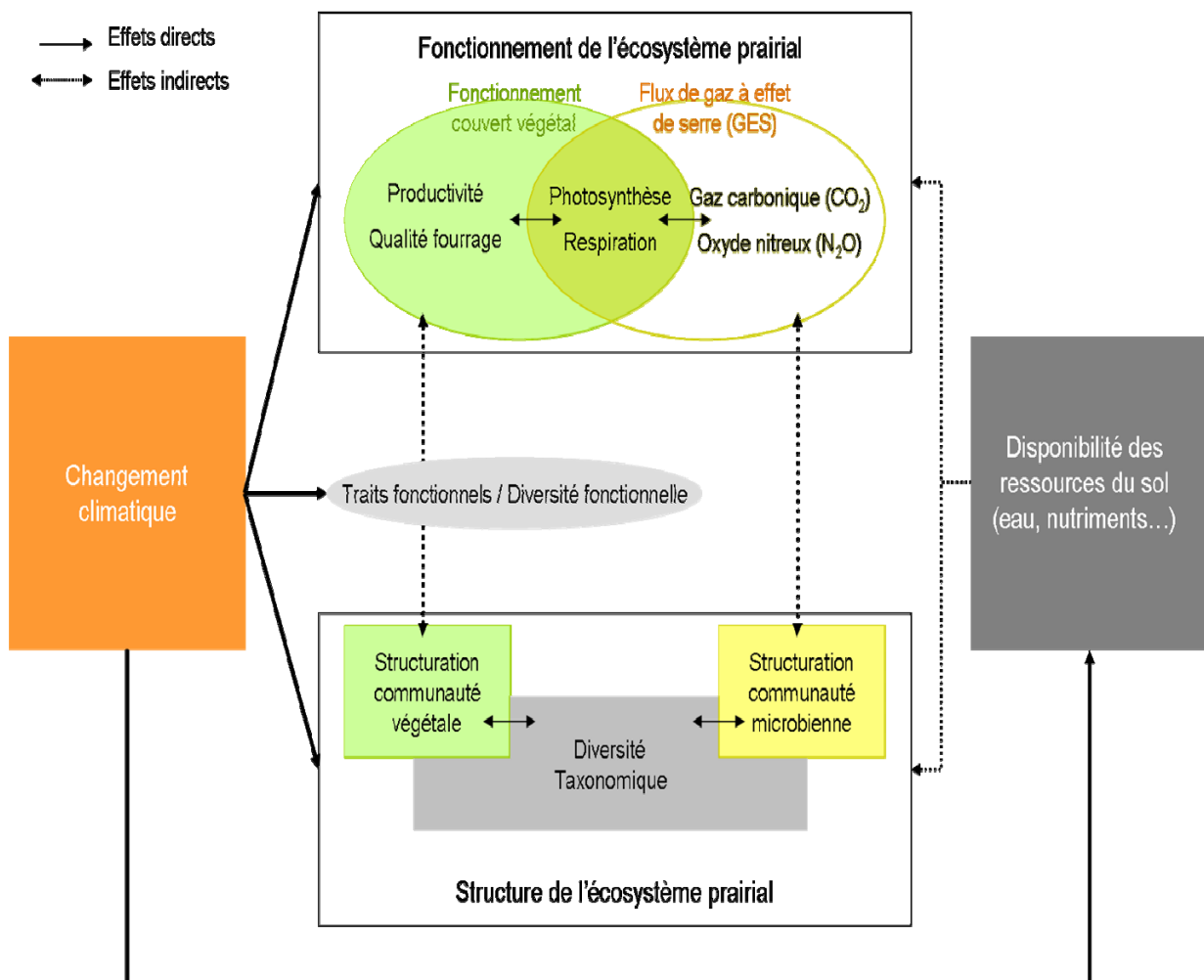


Figure 18 : Cadre conceptuel de la thèse

## 4.1 Dispositif d'étude

Ce travail de thèse s'appuie sur un dispositif expérimental additif *in situ* qui permet de comparer les impacts des principales composantes (température, pluviométrie, CO<sub>2</sub>) du changement climatique sur un assemblage naturel d'espèces prairiales.

Dans le cadre de cette thèse, le scénario retenu pour tester l'impact d'un changement climatique futur sur les écosystèmes est le scénario ACACIA A2. Ce scénario dit scénario 'Entreprise Provinciale', donne la primauté aux groupements nationaux et régionaux, à une croissance de la population forte, avec un développement économique de type protectionniste et une société tournée vers la consommation. Ce scénario centré sur l'Auvergne prévoit à l'horizon 2080 une augmentation de 3.5°C des températures de l'air, une augmentation des concentrations atmosphériques en CO<sub>2</sub> de 200 ppm et une réduction des précipitations estivales de 20 %.

L'écosystème étudié correspond à une prairie permanente sur la commune de Theix (45°43'N, 03°01'E, 850 m a.s.l.; sol brun limono-sableux sur granite) géré expérimentalement depuis 15 ans par un pâturage ovin léger (quatre passages brefs par an) sans fertilisation minérale ou organique. La richesse spécifique initiale de la prairie est faible (15 espèces végétales), dominée par des graminées pérennes et comprenant quelques dicotylédones herbacées (*i.e.* vesce). Des monolithes (blocs de terre et de végétation) de cette prairie permanente ont été prélevés et placés dans des boîtes en acier inoxydable à la taille des monolithes (soit 4 cotés et un fond percé, 50 x 50 x 40 cm de profondeur). A l'automne 2004, puis au printemps suivant, la composition botanique de chaque monolithe a été déterminée. L'analyse de ces données a permis d'allouer les monolithes aux traitements expérimentaux en garantissant l'absence d'effets significatifs des traitements climatiques sur l'écosystème d'étude au temps t0 (Mai 2005) de l'expérience. Cette précaution est cruciale pour la réussite d'expériences de ce type, qui doivent vérifier l'hypothèse nulle d'une homogénéité initiale de la végétation étudiée.

Le dispositif expérimental additif comprend quatre traitements climatiques avec cinq répétitions par traitement (voir encadré 1) :

Le traitement "témoin" (C) dans les conditions climatiques du site de montagne de Theix

(850m).

Le traitement "augmentation de température" (T), + 3 °C obtenu en transportant des monolithes en plaine (Clermont-Ferrand, 45°47'N, 03°05'E, 350m a.s.l., gradient altitudinal de 500 m).

Le traitement "température et sécheresse estivale" (TD), - 20 % de pluie obtenu grâce au climat plus sec en été du site de plaine.

Le traitement "température, sécheresse estivale et enrichissement en CO<sub>2</sub>" (TDCO<sub>2</sub>), + 200 ppm obtenu par fumigation à l'air libre (Mini-FACE) de CO<sub>2</sub> dans des anneaux de 1 m 50 de diamètre.

Les différents traitements ainsi obtenus permettent d'effectuer des analyses statistiques diverses et de tester l'impact de la température (T vs C), de la sécheresse estivale (TD vs T), de l'élévation en CO<sub>2</sub> atmosphérique (TDCO<sub>2</sub> vs TD) et de la combinaison de tous les facteurs (TDCO<sub>2</sub> vs C).

## **4.2 Organisation du manuscrit**

Dans le premier chapitre de résultats (I), la dynamique et le fonctionnement d'un couvert prairial sous changement climatique durant les cinq années de fonctionnement du dispositif seront abordés. Ce chapitre a pour but : 1) d'analyser les liens entre fonctionnement (production de biomasse aérienne) et structure (diversité spécifique et traits fonctionnels) d'un écosystème prairial soumis à un changement climatique, 2) de tester l'importance des effets directs de traitements climatiques sur la production du couvert et d'effets indirects liés aux changements de diversité spécifique et/ou fonctionnelle et/ou des traits fonctionnels. La dynamique de la communauté herbacée a été suivie de 2006 à 2009 en termes de production aérienne, de traits fonctionnels agrégés à la communauté, de composition botanique et de richesse spécifique.



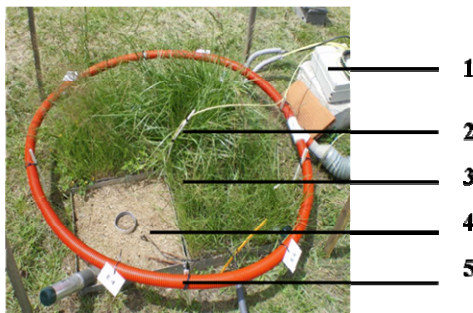
**Encadré 1 : Le dispositif expérimental**

Détail des conditions expérimentales du dispositif. Seules les variables *en italique font* l'objet d'une manipulation.

Traitement	[CO <sub>2</sub> ] (ppm)	Température annuelle (°C)	Pluviométrie estivale (mm)	Site
<b>C</b> : Témoin, conditions ambiantes du site	380	8.8	310	Theix
<b>T</b> : +3,5°C	380	12.1	<i>310</i>	Clermont
<b>TD</b> : +3,5°C, -20 % H <sub>2</sub> O en été	380	12.1	<i>240</i>	Clermont
<b>TDCO<sub>2</sub></b> : +3,5°C, -20 % H <sub>2</sub> O en été, +200ppm CO <sub>2</sub>	<i>580</i>	12.1	<i>240</i>	Clermont



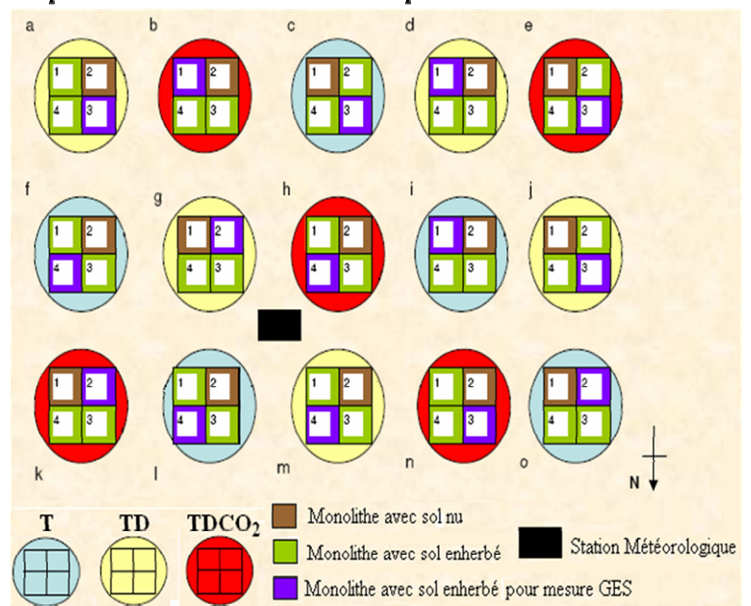
Unité expérimentale ou anneau mini-FACE et ses quatre monolithes; ici enrichit en CO<sub>2</sub>



Légendes

1. Ventilateur pour le soufflage d'air
2. Prélèvement pour le contrôle de la [CO<sub>2</sub>]
3. Monolithe sol enherbé (x 3)
4. Monolithe sol nu (x 1)
5. Anneau creux perforé qui insuffle le CO<sub>2</sub>

Disposition des traitements climatiques sur le site de Clermont



Le chapitre suivant (II) traite de la caractérisation de la végétation en termes de réponses écophysiologicals au changement climatique : échanges de CO<sub>2</sub> (plantes-atmosphère) et allocation des ressources carbonées (*i.e.* étude des transferts de sucres dans les feuilles et les gaines des espèces dominantes) pendant la saison de croissance. Cette caractérisation a fait l'objet d'une étude multi-échelle, partant de l'échelle de l'individu (espèce dominante) à celle de la communauté prairiale. Pour acquérir les techniques en écophysiologicals (études des sucres) une collaboration a été mise en place avec Marie-Pascale Prudhomme, Annette Morvan-Bertrand et Marie-Laure Decau du laboratoire d'Ecophysiologicals Végétale, Agronomie & nutrition NCS de l'université de Caen.

Dans le chapitre III, les dynamiques saisonnières et annuelles des flux de l'oxyde nitreux (N<sub>2</sub>O) en prairies seront analysées, en s'attachant à comprendre comment les facteurs climatiques ou abiotiques (*i.e.* température du sol, humidité du sol, pluviométrie) interagissent dans leurs effets sur les flux de N<sub>2</sub>O. Le chapitre IV des résultats a pour objectif de caractériser les interactions sol-plantes-atmosphère sous conditions de changement climatique par la mise en relation des flux de N<sub>2</sub>O à l'échelle du couvert et de l'écologie microbienne associée (mesures des activités potentielles, quantification de gènes clés dans la production de N<sub>2</sub>O). Ce travail a été réalisé en collaboration avec Franck Poly du laboratoire d'écologie microbienne (LEM) de l'université de Lyon I.

Dans la dernière partie de ce manuscrit, une synthèse des principaux résultats de ce travail est proposée afin d'en dégager les principales perspectives de recherche. Cela permettra de réaliser un bilan sur les apports de ce travail en termes de compréhension des mécanismes de réponse des écosystèmes prairiaux aux changements climatiques futurs.

# Résultats - Chapitre 1

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## **Réponses progressives de la production de biomasse aérienne, de la diversité et des traits fonctionnels en prairies sous changement climatique.**

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Ce chapitre est consacré à l'analyse des liens directs et indirects entre fonctionnement (production de biomasse aérienne) et structure (diversité spécifique et traits fonctionnels) d'un écosystème prairial soumis à un changement climatique durant cinq années. Il fait suite à un premier article publié dans '*Ecosystems*', Bloor *et al.* (2010), qui sera placé en annexe de cette thèse afin de permettre au lecteur une meilleure compréhension du dispositif. Il est présenté sous forme d'un article soumis à la revue '*Ecosystems*' et s'intitule : 'Five years of simulated climate change reduces aboveground productivity and alters functional diversity in an upland grassland.'. Les co-auteurs sont Jean-François Soussana<sup>a</sup> et Juliette Bloor<sup>b</sup>.

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a : INRA - CODIR - ENVIRONNEMENT Paris, France

b : INRA - UR 874 Clermont-Ferrand, France

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## 1- Abstract

Climate change is expected to affect ecosystem productivity both directly and indirectly *via* changes in community structure and plant functional traits. To better understand the effects of climate drivers on ecosystem structure and function, we exposed a perennial, temperate grassland to a simulated climate change corresponding to the air temperature, atmospheric CO<sub>2</sub> and summer rainfall conditions projected for 2080. We examined impacts of climate treatments on aboveground biomass and community structure for five years, and investigated the relationship between biomass production, species diversity and three key functional traits: specific leaf area, leaf dry matter content and leaf nitrogen content. Both warming and simultaneous application of warming, summer drought and elevated CO<sub>2</sub> were associated with an increase in annual aboveground biomass at the start of the study, but biomass responses became progressively negative over the course of the experiment. Although species richness and taxonomic diversity showed no response to climate treatments, the relative abundance of graminoid and legumes showed significant effects of both warming and simultaneous application of warming, summer drought and elevated CO<sub>2</sub> after three years. Functional diversity responses varied depending on climate treatment and leaf trait. Overall, patterns of variation in annual plant biomass were best explained by community-level leaf traits. Taken together, our data suggest that continuous, multi-year exposure to projected climate conditions will have a negative impact on aboveground biomass in our grassland study system. Limited response of species diversity to climate change in the short term may reflect increased phenotypic plasticity in leaf functional traits.



## 2- Introduction

Continued greenhouse gas emissions at or above current rates are expected to cause multiple and co-occurring changes in global temperatures and rainfall patterns, but considerable uncertainty remains over the impacts of simultaneous environmental changes on the major biomes worldwide (IPCC 2007). Understanding the impacts of rising atmospheric CO<sub>2</sub>, global warming and changes in precipitation patterns on grassland ecosystems is of particular importance since this terrestrial biome covers more than a quarter of the total ice free land area and provides ecosystem goods and services at a global-scale (Gibson 2009).

Over the last decade, the number of grassland climate change experiments involving multiple and simultaneous climate change drivers has shown a significant increase (Dukes *et al.* 2005; Hovenden *et al.* 2008; Grime *et al.* 2008; Sherry *et al.* 2008; Bloor *et al.* 2010). For the most part, these studies have been of relatively short duration (3 years or less, but see Dukes *et al.* 2005; Grime *et al.* 2008; Kardol *et al.* 2010), with little consistency in climate change treatments across studies. It is perhaps unsurprising then, that grassland production responses to climate change drivers (temperature, rainfall or elevated atmospheric [CO<sub>2</sub>]) vary widely among study sites and years (reviewed by Dukes *et al.* 2005; Bloor *et al.* 2010). Plant-soil feedbacks, threshold responses and temporal lags in plant growth or mortality may complicate the prediction of biomass responses to long-term environmental changes (Shaver *et al.* 2000; Dunne *et al.* 2004). For example, elevated [CO<sub>2</sub>] may reduce soil nitrogen (N) availability resulting in declines in aboveground productivity over time, a process termed as progressive N limitation (PNL *sensu* Luo *et al.* 2004). However, recent work has shown that PNL may be overcome by warming-induced increases in N availability (Hovenden *et al.* 2008), and the importance of progressive climate effects for grasslands exposed to changes in multiple climate drivers remains unclear.

Climate-induced changes in biomass production can result from direct effects on plant physiological processes or indirect effects via changes in community structure (Lavorel & Garnier 2002). Such shifts in plant community structure, generally associated with changes in soil processes, plant-soil and plant-plant interactions, are often expressed in terms of species richness, species evenness or presence and relative abundance of plant functional types i.e. legumes, forbs, graminoids (Marquard *et al.* 2009). Modifications in grassland

species richness and relative abundance of such functional types have previously been demonstrated for elevated [CO<sub>2</sub>] (Owensby *et al.* 1993; Teyssonneyre *et al.* 2002; Zavaleta *et al.* 2003; Niklaus & Körner 2004), warming (Grime 2001; Zavaleta *et al.* 2003; De Boeck *et al.* 2008) and drought (Engel *et al.* 2009), although evidence for changes in response to combined climate change drivers is mixed (Zavaleta *et al.* 2003; Grime *et al.* 2008; Engel *et al.* 2009; Bloor *et al.* 2010). More recently, attention has also been drawn to the role of plant trait variability and functional diversity (defined as the value, range and relative abundance of plant functional traits present in a given community, de Bello *et al.* 2010) in modulating ecosystem responses to environmental change (Diaz *et al.* 2007; Suding *et al.* 2008).

Plant functional traits are of interest because of their potential to: i) impact on plant fitness *via* effects on growth, reproduction or survival, ii) vary in response to environmental conditions/ resource availability ('response traits'), and iii) affect ecosystem processes such as nutrient cycling and below-ground carbon sequestration ('effect traits') (Lavorel & Garnier, 2002; Violle *et al.* 2007; Klumpp & Soussana, 2009). Plasticity in key functional traits such as specific leaf area (SLA) and leaf nitrogen concentration (LNC) are well established in response to elevated CO<sub>2</sub> (Chapin *et al.* 1993; Grime 2001; Wright *et al.* 2004), and have also been documented in response to warming (Hudson *et al.* 2010). However, data on functional diversity in grassland climate change studies is scarce. It has been suggested that functional diversity should respond more rapidly to climate change than taxonomic diversity, and that the relative importance of these components of community structure for ecosystem function should vary over time (Suding *et al.* 2008), but this has yet to be demonstrated experimentally.

Here we investigate the effects of warming, summer drought and elevated [CO<sub>2</sub>] on a perennial temperate grassland, using climate manipulations based on 2080 climate projections for the study area (ACACIA A2 scenario for central France, IPCC 2001). Preliminary results from this *in situ* grassland experiment indicated an initial warming-induced stimulation in spring biomass production (Bloor *et al.* 2010). In the present study, we examine the dynamics of annual aboveground production, plant community structure and three key functional leaf traits under warming and combined climate change treatments over a five-year period. We determine whether the initial stimulation of production by warming persists in the longer term and ask the following questions: (1) Is there any evidence for progressive responses of aboveground biomass production to climate treatments?; (2) Do taxonomic diversity and



functional diversity (assessed by divergence in leaf traits) differ in their sensitivity to climate change drivers?; (3) What are the respective roles of taxonomic diversity and of changes in community-scale leaf traits in mediating the observed responses in above-ground production?

### 3- Materials and methods

#### 3.1 Experimental site and climate change treatments

The Clermont Climate Change Experiment is located in the French Massif Central region near Clermont Ferrand (45°47'N, 03°05'E). This region experiences a continental climate with cold winters (much of the water inputs occurring as snow) and relatively dry, hot summers. The experiment was conducted using grassland monoliths extracted from an extensively-managed upland semi-natural grassland site (Theix, 45°43'N, 03°01'E, 850 m a.s.l.) with a mean annual temperature of 8.7 °C and annual precipitation of 780 mm. The soil in this location is characterised as a Cambisol soil (59.5 % sand, 19.7 % silt, 20.8 % clay, pH<sub>H2O</sub> 6.2) and the grassland community is dominated by grasses including *Elytrygia repens*, *Festuca arundinacea*, *Festuca rubra*, *Agrostis capillaris*, *Arrhenatherum elatius*, *Dactylis glomerata*, *Poa pratensis* and *Trisetum flavescens*. Forbs and legumes are present (*Veronica chamaedrys*, *Vicia sativa*, *Vicia hirsuta*, *Stellaria media*, *Taraxacum officinalis*) but rarely dominant.

The experiment was established in 2005, manipulating three drivers of climate change (temperature, drought and atmospheric CO<sub>2</sub>) in a replicated, additive design (see Bloor *et al.* 2010 for full details). In brief, 80 grassland monoliths (0.5 x 0.5 x 0.4 m deep) were extracted in November 2004 using a bespoke turf-cutting system and encased individually in stainless-steel boxes with 81 drainage holes (1.5 cm diameter) in the bottom. Monoliths were left for 6 months on-site to acclimate to their boxes in specially prepared trenches, surrounded by compacted soil. In late April 2005, monoliths were lifted from their trenches and assigned to one of four climate treatments; C (control, original site conditions), T (+3.5 °C), TD (+3.5 °C, 20 % reduction in summer rainfall) and TDCO<sub>2</sub> (+3.5 °C, 20 % reduction in summer rainfall, +200 ppm CO<sub>2</sub>). The TDCO<sub>2</sub> treatment was based on IPCC forecasts for the French Massif Central region for 2080 (ACACIA, A2 scenario, IPCC 2001), and the additive treatment combinations provided a cost-effective and efficient approach for examining grassland

responses to warming and to combined drivers of climate change (elevated CO<sub>2</sub>, warming and rainfall pattern).

In each of the four climate treatments, we established five replicated experimental units each consisting of four monoliths. All experimental units were lowered into the ground in holes lined with a 0.5 m-deep layer of volcanic gravel and a 0.4 m layer of ‘deep’ soil (0.4–0.8 m) collected from the field where monoliths were extracted. Monoliths in the control treatment were left at the upland grassland site at Theix, whereas elevated temperature conditions were obtained by transplanting the remaining monoliths to a lower altitude site at Clermont Ferrand (350 m a.s.l., mean annual temperature of 12.4 °C, mean annual rainfall of 575 mm). Unlike active heating devices (heat resistance cables, overhead infrared lamps) or passive warming by greenhouses/ chambers, spatial climatic gradients have the advantage of simulating warming in a realistic manner with less unintentional, confounding effects on the microclimate (Dunne *et al.* 2004).

Mini-FACE (Free Air Carbon dioxide Enrichment, Miglietta *et al.* 2001) technology was used to elevate atmospheric [CO<sub>2</sub>] to 600 ppm. This target CO<sub>2</sub> concentration was maintained during daylight hours via an automated control system (see Bloor *et al.* 2010 for full description). Briefly, 1.5 m-diameter rings (corrugated polyethylene vent pipes) were used to release CO<sub>2</sub>-enriched air around each TDCO<sub>2</sub> unit, and 1.5m diameter rings blowing ambient air were set up around all remaining experimental units (C, T, and TD treatments) to avoid confounding effects of increased air flow over the plant canopy. All experimental units were placed 7 m apart to ensure that there was no risk of atmospheric contamination between CO<sub>2</sub>-enriched and ambient-CO<sub>2</sub> units. Given consistently lower monthly rainfall levels at the lowland site ( $-18.3 \pm 4.1$  mm), rainfall for monoliths in the lowland site was supplemented by irrigation to generate matching rainfall patterns with the control, upland site. Summer drought in the TD and TDCO<sub>2</sub> treatments was achieved using a combination of rainscreens and watering during June, July and August (see full description in Bloor *et al.* 2010). The plant canopy in the experimental units was cut twice a year (see plant production measurements described below) and no fertiliser was applied, in line with management practices at the upland site.

A Campbell Scientific automatic weather station logged to a CRX-10 data logger at half-hourly intervals (Campbell Scientific Inc, Utah, USA) was used to record climatic data at

the upland (C treatment) and lowland sites (T, TD, TDCO<sub>2</sub> treatments). Potential evapotranspiration (PET, mm month<sup>-1</sup>) at the two sites was calculated using the Penman-Monteith equation (FAO, 2005), and the ratio of cumulated monthly rainfall to cumulated monthly PET was used as an index of water balance. Volumetric soil water content (SWC) was measured every 30 minutes for three replicates per climate treatment using Echo dielectric capacity probes vertically inserted into soil (0-20 cm) and connected to a datalogger (Ech<sub>2</sub>O, Decagon Devices, USA). The relationship between SWC<sub>g</sub> and SWC was established as:  $SWC_g = 0.0948 \ln(SWC) + 0.4021$  using probe measurements in 5 l pots filled with soil ( $n = 108$ ,  $R^2 = 0.81$ ; Picon-Cochard, personal communication). Soil water potential was determined from gravimetric soil water content (SWC<sub>g</sub>) measurements at field capacity (0.03 MPa) and at wilting point (1.5 MPa) with a dewpoint meter (PotentialMeter WP4, Decagon Devices, USA).

### **3.2 Data collection**

One year after the start of the experiment (April 2006), biomass harvests were initiated to determine annual aboveground biomass production. Two aboveground biomass harvests were carried out per year at 6-month intervals (late April and late October), corresponding roughly to the start and end of the plant growing season in an average year at the control site; harvests in late April occurred 2-3 weeks after re-greening at the control site and are consistent with local animal turn-out dates. At each harvest, vegetation was cut to a height of 5 cm. Biomass from the 5 cm strip around the perimeter of each monolith was discarded to avoid edge effects, and the collection of biomass data was restricted to the central 0.4 x 0.4 m zone of each monolith. A subsample of 30-40 g freshly-harvested material was sorted into green and senescent material. Green biomass was separated to species, and all biomass was oven dried (60 °C, 48 h) before weighing. Annual biomass production was estimated by summing the values from the two biomass harvests per experimental unit per year. Species biomass data were used to determine abundance of functional groups (legumes, forbs, graminoids), as well as Shannon-Weaver diversity and equitability indices (Kent & Coker, 1992). A second subsample of 15-20 g was sorted into green and senescent material and was used to determine exported nitrogen (N) in green and senescent biomass using an elemental analyser (Carlo Erba Instruments, CNS NA 1500 ThermoFinnigan, Milan, Italy). Annual N exports by clipping were estimated from 2006 onwards by summing the N values from green and senescent biomass for the two harvests per experimental unit.

Point quadrat measurements (16 points per monolith across the central zone 0.4 x 0.4 m) were used to determine the plant community structure prior to each April harvest. Immediately after, three vegetative leaf traits were measured for dominant plant species (contributing to 5% or more of the cover) by harvesting 10 tillers chosen at random across each experimental unit. In the laboratory, the tiller base was cut in de-ionized water and was then placed at 4°C in the dark for at least 6 h to allow for full rehydration (Garnier *et al.* 2001). After rehydration, the lamina of the youngest fully expanded leaf of each of the ten individuals was measured, weighed and their area was measured with an electronic planimeter (LI 3100, Li-cor, Lincoln, NE, USA). The leaves were then oven dried at 60°C for 48 h and weighed. The traits measured were: i) specific leaf area (SLA; leaf lamina area/leaf lamina dry mass, critical for plant-atmosphere gas exchange), ii) leaf dry matter content (LDMC, leaf lamina dry mass/leaf lamina fresh mass; index of leaf tissue density, related to plant nutrient retention and growth) and LNC (leaf N content, integral to proteins involved with photosynthesis) (Wright *et al.* 2004). For the LNC determination, dried leaf samples of each species were ground using a sample mill (Mixer Mill, Model MM200; Retsch GmbH, Haan, Germany) and analysed with an elemental analyser (Carlo Erba Instruments, CNS NA 1500 ThermoFinnigan, Milan, Italy). Community-level trait values were calculated as a weighted average for each experimental unit using the relative contribution of each species to the total above ground biomass at the corresponding April harvest. Functional diversity was determined using Rao's index, where the degree of overlap of trait values within the community is calculated from species' relative abundance and individual species' leaf traits (Lepš *et al.* 2006).

### 3.3 Data analysis

Effects of climate treatment on annual plant biomass, functional groups, species diversity indices and community-level leaf traits were analysed using mixed model repeated measures analysis of variance (ANOVA) and mean values per variable per experimental unit. All percentage data (functional group abundance, leaf nutrient content) were transformed by the arcsine function to conform with assumptions of normality. Effects of individual climate change drivers (temperature, drought, CO<sub>2</sub>) were analysed using orthogonal contrasts (see Gilligan 1986). Effects of warming were determined by comparing the C and T treatment; effects of summer drought by comparing T and TD; effects of elevated [CO<sub>2</sub>] by comparing TD and TDCO<sub>2</sub>; effects of simultaneous application of warming, summer drought, and CO<sub>2</sub>

enrichment (2080 climate scenario) were investigated by the C versus TDCO<sub>2</sub> comparison. Given that our warming treatment is not replicated in a statistical sense, we assume that any differences C and T are driven primarily by the large temperature difference between lowland and upland sites (Dunne *et al.* 2004). Correlation analysis between annual plant biomass and species/functional diversity indices was performed using mean values of community-level leaf traits (SLA, LDMC, LNC), functional diversity of leaf traits (FD<sub>SLA</sub>, FD<sub>LDMC</sub>, FD<sub>LNC</sub>), species diversity (richness, Shannon-Weaver, evenness) and log-transformed annual biomass production values per climate treatment for all years. All analyses were carried out using Statgraphics Plus 4.1 (Statistical Graphics Corp., Rockville, Maryland, USA).

## 4- Results

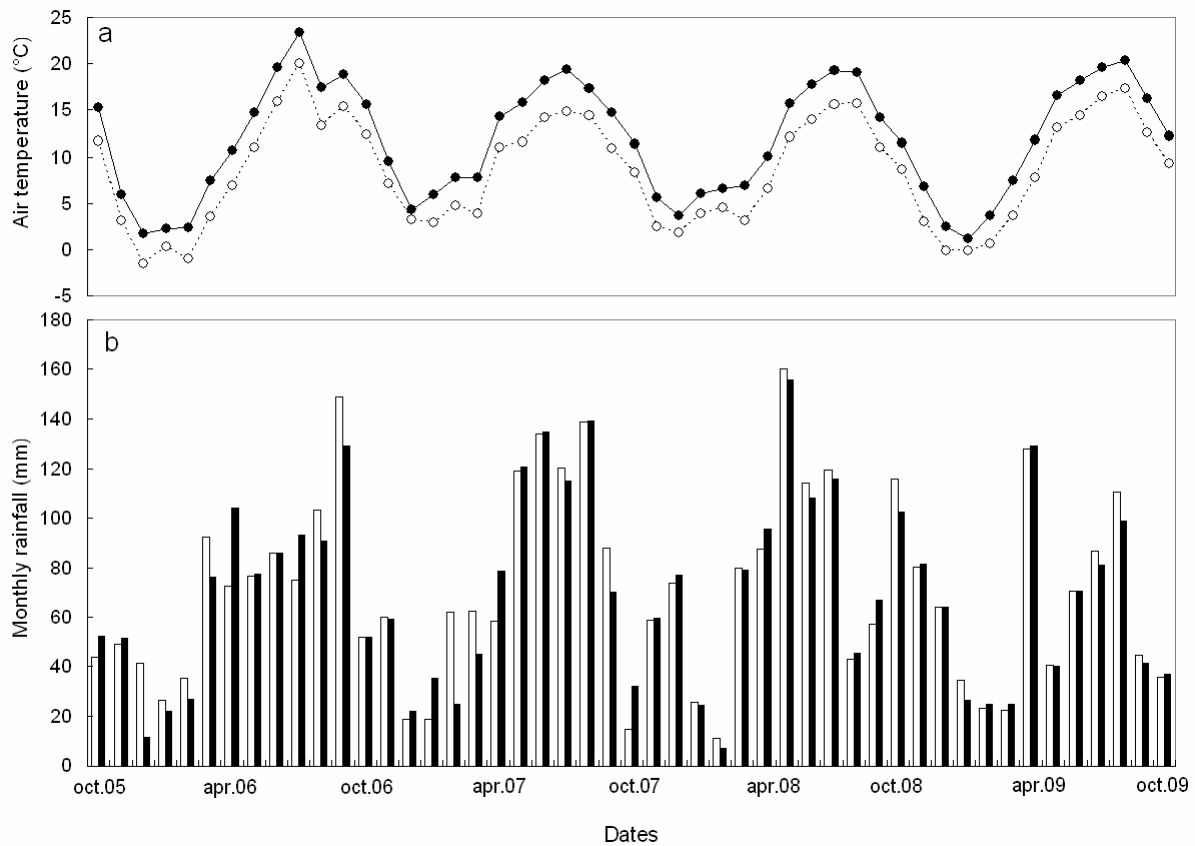
### 4.1 Climate

From 2006 to 2009, the difference in mean monthly temperature between control and elevated temperature treatments was  $3.3 \pm 0.11$  °C (monthly range between 1.2 and 4.5 °C, Figure 1). Mean monthly precipitation between C and T treatments showed no significant difference over the study (repeated measures ANOVA,  $F_{1,3} = 0.45$ ,  $P > 0.05$ ; Figure 1). Nevertheless, soil water content was significantly higher in the C compared with the T treatment throughout the study period (repeated measures ANOVA,  $F_{1,3} = 146.3$ ,  $P < 0.001$ ; Figure 2). PET was lower in C than T treatment during the study (mean difference in annual PET of  $-104 \pm 20$  mm), despite increased wind exposure at the upland site (monthly mean windspeed of 2.2 m and  $1.4 \text{ m s}^{-1}$  at the upland and lowland sites, respectively, Student's  $t = -7.89$ ,  $P < 0.001$ ). No significant differences were recorded between the control and warmed site in terms of monthly global radiation levels ( $P > 0.05$ ).

During the summer rainfall reduction period, the drought treatments (TD, TDCO<sub>2</sub>) received a  $21.9 \pm 0.55$  % reduction in rainfall compared with the T treatment (Figure 1). Despite rainfall reduction, summer soil moisture values for TD were only significantly lower than T in 2007 and 2009 (repeated measures ANOVA,  $F_{1,3} = 7.88$ ,  $P < 0.05$  and  $F_{1,3} = 8.01$ ,  $P < 0.05$  respectively, Appendix 1). Mean daily values of CO<sub>2</sub> in the elevated [CO<sub>2</sub>] treatment (TDCO<sub>2</sub>) reached  $585 \pm 1.4$  ppm *versus*  $380 \pm 3.8$  ppm recorded in ambient CO<sub>2</sub> conditions. No significant differences in ambient CO<sub>2</sub> values were found between the upland and lowland

sites during the study period. Furthermore, soil moisture values showed no significant difference between TD and TDCO<sub>2</sub> throughout the study (Figure 2).

Comparisons with long term meteorological observations at the control site (1986-2003) indicated that rainfall was above average from 2006-2008, but slightly below average in 2009. Mean daily air temperatures for the growing season were close to the long-term average during the study, although winters in 2006 and 2009 were characterised by below-average temperatures.

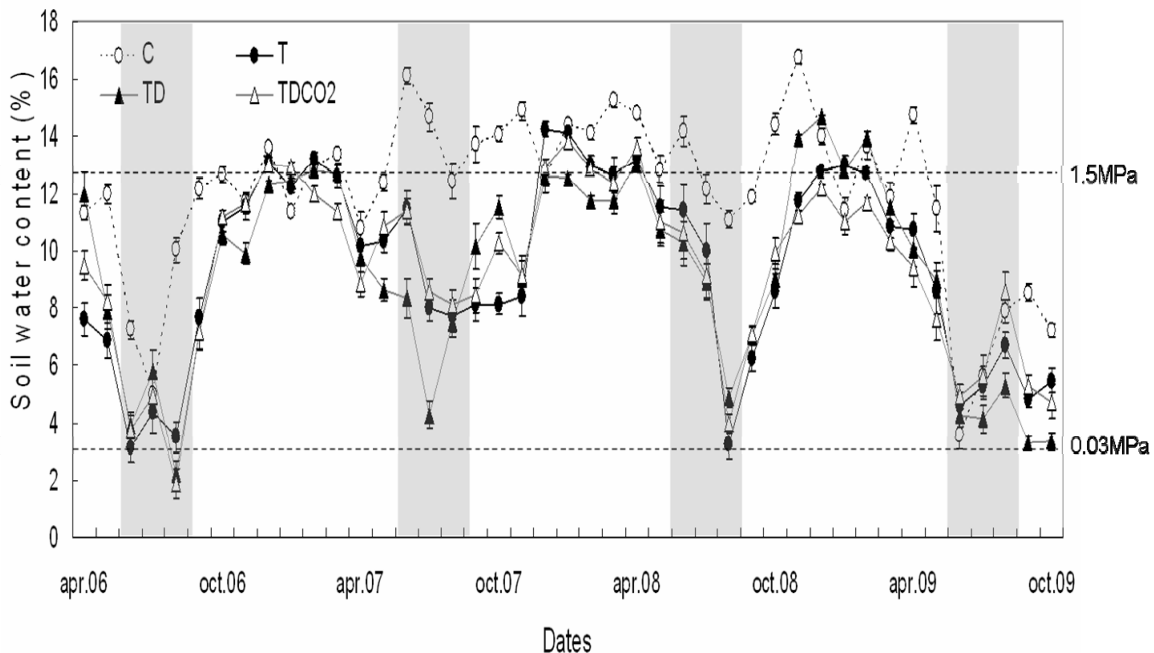


**Figure 1. Monthly averages of a) air temperature and b) rainfall for the control and elevated temperature sites during the experimental period. The control, upland site is given by open symbols (circles or bars) whereas the warmer, lowland site is presented by filled symbols.**

#### 4.2 Annual aboveground biomass production, and exported N

Annual aboveground plant biomass showed significant effects of treatment and year (repeated measures ANOVA,  $F_{1,3} = 3.7$ ,  $P < 0.05$  and  $F_{1,3} = 30.4$ ,  $P < 0.001$  respectively) but effects of climate drivers varied over time (significant Treatment x Year interaction;  $F_{1,16} = 5.33$ ,  $P < 0.01$ ; Figure 3). Warming (C versus T comparison) had a positive effect on annual biomass

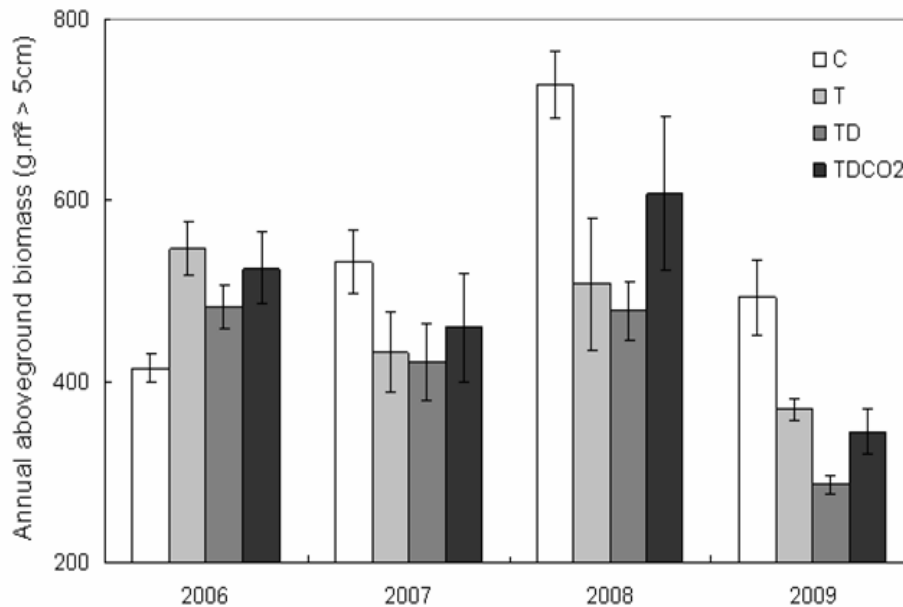
production in 2006 (+32 %,  $F_{1,16} = 13.5$ ,  $P < 0.01$ ), no significant effect in 2007 and a significant negative effect in 2008 and 2009 (-30 %,  $F_{1,16} = 9.2$ ,  $P < 0.05$  and -24 %,  $F_{1,16} = 26.6$ ,  $P < 0.001$  respectively). This pattern of response was also found for annual plant biomass in the combined climate change treatment (C vs TDCO<sub>2</sub>): a 26.6% increase in 2006 ( $F_{1,16} = 10.39$ ,  $P < 0.05$ ); no treatment effect in 2007; a significant negative effect in 2008 and 2009 (-16.5 %,  $F_{1,16} = 7.68$ ,  $P < 0.05$  and -30 %,  $F_{1,16} = 28.11$ ,  $P < 0.001$  respectively). Consequently, both warming and combined climate change were associated with a progressive decrease in annual biomass production over time (negative correlation between treatment effects and time,  $r = -0.72$ ,  $P < 0.001$  and  $r = -0.70$ ,  $P < 0.001$  for warming and combined climate change respectively).



**Figure 2.** Mean monthly soil water content (%) for experimental treatments during the study. Treatments are given by: C = Control; T = elevated temperature; TD = elevated temperature and summer drought; TDCO<sub>2</sub> = elevated temperature, summer drought and CO<sub>2</sub> enrichment. Summer rainfall reduction periods for the TD and TDCO<sub>2</sub> treatments are shaded in grey. Horizontal lines correspond to values of soil water potential measured at field capacity (0.03MPa) and at wilting point (1.5MPa).

Unlike warming and combined climate change, effects of summer drought (T vs TD) and elevated CO<sub>2</sub> (TD vs TDCO<sub>2</sub>) on annual aboveground biomass did not switch from positive to negative during the study period. In general, annual biomass showed a tendency towards lower values in response to summer drought, and a tendency towards higher values in response to increased CO<sub>2</sub> (Figure 3). However, drought was only associated with a significant negative effect on annual biomass in 2009 (-22.7 %,  $F_{1,16} = 14.4$ ,  $P < 0.01$ ). Effects

of CO<sub>2</sub> were significant in 2008 and 2009 (+ 27.4 %,  $F_{1,16} = 6.8$ ,  $P < 0.05$  and +20.5 %,  $F_{1,16} = 15.8$ ,  $P < 0.01$  in 2008 and 2009 respectively).

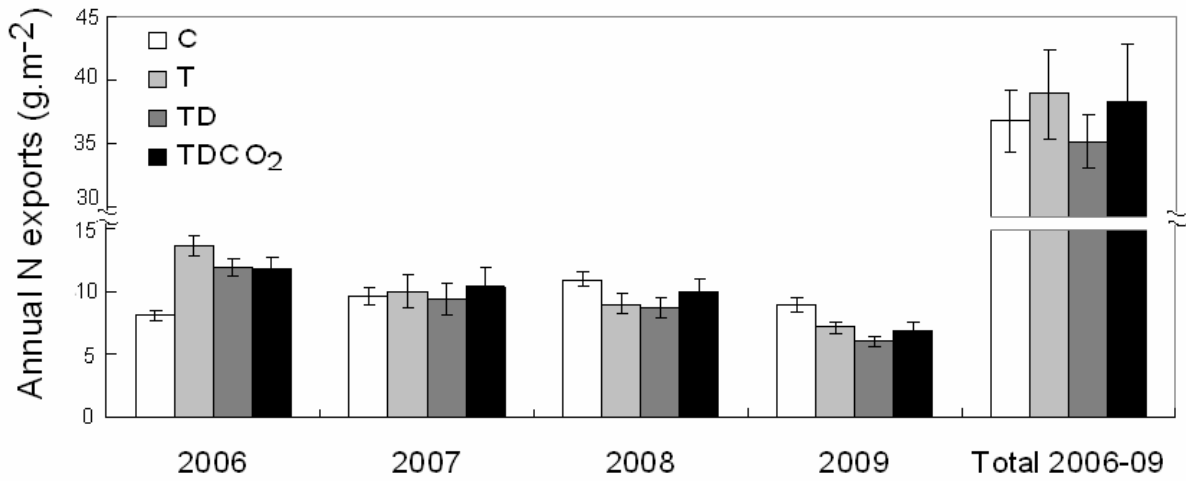


**Figure 3: Effects of climate manipulations on annual plant biomass. Treatments are given by: C = Control; T = elevated temperature; TD = elevated temperature and summer drought; TDCO<sub>2</sub> = elevated temperature, summer drought and CO<sub>2</sub> enrichment. Means, standard errors are presented per treatment and year (n=5)**

Effects of climate treatment on annual exported N generally mirrored patterns observed for aboveground biomass (Figure 4). Both climate treatment and year had a significant effect on the mass of nitrogen (N) exported by harvests, but the effect of treatment varied between years (significant Treatment x Year interaction,  $F_{1,16} = 5.03$ ,  $P < 0.01$ ). Warming had a positive effect on annual N exports in 2006 (+68.2 % for T vs C,  $F_{1,16} = 37.5$ ,  $P < 0.001$ ). No significant warming effects were found in 2007 and 2008, but a negative effect of warming was found in 2009 (-20.5 %,  $F_{1,16} = 13.2$ ,  $P < 0.01$ ; Figure 4). Similar trends were also found for annual N exports in response to the simultaneous application of warming, drought and CO<sub>2</sub> (Figure 4). Annual N exports showed a significant decrease in response to summer drought in 2006 and 2009 (T vs TD, -12.5 %,  $F_{1,16} = 12.6$ ,  $P < 0.01$  and -



16 %,  $F_{1,16} = 7.1$ ,  $P < 0.05$ , respectively). In addition, elevated  $CO_2$  had a positive impact on annual N exports in 2009 (+15.4 %,  $F_{1,16} = 7.5$ ,  $P < 0.05$ ).



**Figure 4:** Effects of climate manipulations on annual N exports for 2006-09 and total N export over the experimental period. Treatments are given by: C = Control; T = elevated temperature; TD = elevated temperature and summer drought; TDCO<sub>2</sub> = elevated temperature, summer drought and CO<sub>2</sub> enrichment. Means and standard errors are presented per treatment and year ( $n = 5$ ).

#### 4.3 Plant community composition.

During the study period, we identified  $6.9 \pm 0.1$  species on average per experimental unit (range from 4 to 12 species) based on biomass collected at the April harvests. The number of species showed no significant variation from 2006 to 2009 ( $P > 0.05$ ). Grass species dominated the plant communities in the experimental units across treatments and years (Table 1). However, the proportion of graminoids to non-graminoids showed a steady decline across treatments over the study period, decreasing from  $97.1 \pm 1.2$  % of the plant community composition in 2006 to  $86.4 \pm 3.3$  % in 2009 ( $F_{1,79} = 4.38$ ,  $P < 0.01$ ). In contrast, the proportion of legumes increased significantly over time from  $0.2 \pm 0.1$  % of the community biomass in 2006 to  $7.9 \pm 2.2$  % in 2009 ( $F_{1,79} = 18.85$ ,  $P < 0.001$ ). Forb proportions remained stable over time.

The relative abundance of functional types showed stronger responses to warming (C versus T) than to any other climate treatment, but the effects of warming varied depending on both functional type and year. No significant warming effects were observed in the first years of the experiment. However, in both 2008 and 2009, warming had a negative effect on grass abundance ( $F_{1,16} = 13.5$ ,  $P < 0.01$  and  $F_{1,16} = 6.12$ ,  $P < 0.05$ , respectively; Table 1) and a positive impact on legume abundance ( $F_{1,16} = 6.12$ ,  $P < 0.05$  and  $F_{1,16} = 5.12$ ,  $0.1 < P < 0.05$ ,

respectively). Effects of warming on grass abundance showed a significant negative correlation with time ( $r = -0.59$ ,  $P < 0.01$ ), but there was no significant relationship between warming effects on legume abundance and time. Combined climate change had a negative impact on grass abundance in 2008 and 2009 ( $F_{1,16} = 17.47$ ,  $P < 0.01$  and  $F_{1,16} = 5.49$ ,  $0.1 < P < 0.05$ , respectively) and a positive impact on legumes in 2008 ( $F_{1,16} = 5.71$ ,  $0.1 < P < 0.05$ ), but no progressive changes were observed with time. Neither summer drought (T vs TD), nor elevated CO<sub>2</sub> (TD vs TDCO<sub>2</sub>) had any significant effect on the relative abundance of grasses and legumes during the study. In addition, climate change treatments had no significant effect on forb abundance throughout the study ( $P > 0.05$  in all cases). Despite changes in functional group abundance, neither species richness, species diversity nor equitability showed any significant response to climate treatments over the five-year study period ( $P > 0.05$  in all cases; data not shown).

**Table 1. Effects of climate change treatment on the relative contribution (%) of functional types to community biomass over the experiment**

	Climate Treatments			
	C	T	TD	TDCO <sub>2</sub>
<b>(a) Grasses (%)</b>				
2006	99.1 ± 1.61	98.2 ± 1.76	96.7 ± 2.03	94.3 ± 5.57
2007	97.3 ± 2.11	96.1 ± 1.87	91.8 ± 4.78	93.2 ± 3.58
2008	99.9 ± 1.31	92.8 ± 2.86	87.8 ± 4.38	85.7 ± 6.40
2009	97.9 ± 1.04	88.5 ± 3.26	79.1 ± 5.83	80.1 ± 10.3
<b>(b) Legumes (%)</b>				
2006	0.05 ± 0.05	0.41 ± 0.36	0.27 ± 0.16	0.18 ± 0.15
2007	1.70 ± 1.23	3.08 ± 1.59	2.48 ± 1.59	3.19 ± 2.91
2008	0 ± 0	6.95 ± 2.24	8.35 ± 3.54	6.20 ± 2.87
2009	0.66 ± 0.65	10.7 ± 4.04	10.5 ± 5.35	9.85 ± 7.11
<b>(c) Forbs (%)</b>				
2006	0.89 ± 0.48	1.32 ± 0.98	2.95 ± 1.36	5.44 ± 5.29
2007	1.01 ± 0.63	0.89 ± 0.67	5.76 ± 5.61	3.56 ± 1.96
2008	0.04 ± 0.04	0.20 ± 0.11	3.76 ± 3.51	8.11 ± 7.01
2009	1.34 ± 1.06	0.74 ± 0.54	10.4 ± 5.18	10.1 ± 9.01

#### 4.4 Community-level leaf traits and functional diversity

Both community-level leaf traits and trait functional diversity showed significant responses to climate treatments during the study (Table 2). Climate treatment effects for leaf dry matter content (LDMC) and leaf nitrogen content (LNC) were driven by significant trait responses to

the combined climate change treatment (C vs TDCO<sub>2</sub>;  $F_{1,38} = 5.53$ ,  $P < 0.05$  for LDMC; and  $F_{1,38} = 6.49$ ,  $P < 0.01$  for LNC); specific leaf area (SLA) showed no response to combined climate change. Overall, combined climate change was associated with an increase in LDMC but a decrease in LNC throughout the experiment (Table 2). None of the community-level leaf traits measured showed a significant response to individual climate drivers i.e. warming, drought under warming or CO<sub>2</sub> under warming and drought (C vs T, T vs TD and TD vs TDCO<sub>2</sub> comparisons respectively).

**Table 2. Effects of climate change treatment on community aggregated leaf traits and functional diversity. Means and standard errors are presented ( $n = 20$ ); results from repeated measures ANOVA are shown (\*, \*\*, \*\*\* indicates significant differences at  $P < 0.05$ ,  $<0.01$ , or  $<0.001$ , respectively).**

	Aggregated leaf traits			Functional divergence (RAO index)		
	SLA	LDMC	LNC	FD SLA	FD LDMC	FD LNC
<i>(A) Means (<math>\pm</math> SE)</i>						
C	174 $\pm$ 13.3	26.9 $\pm$ 1.66	3.13 $\pm$ 0.22	0.31 $\pm$ 0.03	0.17 $\pm$ 0.02	0.36 $\pm$ 0.02
T	178 $\pm$ 10.9	28.2 $\pm$ 0.96	3.13 $\pm$ 0.15	0.30 $\pm$ 0.03	0.33 $\pm$ 0.03	0.32 $\pm$ 0.03
TD	174 $\pm$ 11.8	28.8 $\pm$ 1.43	3.08 $\pm$ 0.19	0.36 $\pm$ 0.04	0.27 $\pm$ 0.02	0.37 $\pm$ 0.05
TDCO <sub>2</sub>	167 $\pm$ 12.1	29.5 $\pm$ 1.35	2.72 $\pm$ 0.15	0.28 $\pm$ 0.03	0.33 $\pm$ 0.03	0.21 $\pm$ 0.05
<i>(B) Statistical significance</i>						
Treatment	ns	*	**	*	***	**
Year	ns	***	ns	***	***	***
Treatment x Year	ns	ns	ns	ns	ns	ns

Functional diversity in SLA, LDMC and LNC (FD<sub>SLA</sub>, FD<sub>LDMC</sub> and FD<sub>LNC</sub>) showed significant effects of treatment and years, and effects of treatment were also consistent across time (non-significant Treatment x Year interaction, Table 2). Across treatments, functional diversity in SLA, LDMC and LNC showed a significant increase between 2006/2007 and 2008/2009 (repeated measures ANOVA;  $F_{1,3} = 23.58$ ,  $P < 0.001$ ;  $F_{1,3} = 14.47$ ,  $P < 0.01$ ; and  $F_{1,3} = 19.50$ ,  $P < 0.01$  for FD<sub>SLA</sub>, FD<sub>LDMC</sub> and FD<sub>LNC</sub> respectively). Both warming (C vs T) and combined climate change (C vs TDCO<sub>2</sub>) had a positive effect on FD<sub>LDMC</sub> ( $F_{1,38} = 22.12$ ,  $P < 0.001$  and  $F_{1,38} = 22.24$ ,  $P < 0.001$  respectively; Table 2). In contrast, both elevated CO<sub>2</sub> (TD vs TDCO<sub>2</sub>) and combined climate change (C vs TDCO<sub>2</sub>) treatments had a negative effect on FD<sub>LNC</sub> ( $F_{1,38} = 18.49$ ,  $P < 0.05$  and  $F_{1,38} = 16.28$ ,  $P < 0.05$ ). Values of FD<sub>SLA</sub> also decreased in response to elevated CO<sub>2</sub> (TD vs TDCO<sub>2</sub>,  $F_{1,38} = 5.76$ ,  $P < 0.05$ ), whereas summer drought

alone (T vs TD) had no significant effect on functional diversity in any of the leaf traits measured (Table 2).

Irrespective of climate treatment, community-level leaf trait values showed stronger correlations with annual aboveground biomass across years than did values of functional diversity (Table 3). During the study, annual aboveground biomass showed a positive correlation with SLA and LDMC in the control treatment, but a negative correlation with LDMC and/or SLA in all warmed treatments (T, TD, TDCO<sub>2</sub>). Neither LNC, FD<sub>LNC</sub> nor FD<sub>SLA</sub> were significantly correlated with aboveground biomass in any climate treatment. However, the functional diversity index FD<sub>LDMC</sub> did show significant correlations with annual biomass (Table 3). Aboveground biomass showed a positive correlation with FD<sub>LDMC</sub> in the control treatment, but a negative correlation with FD<sub>LDMC</sub> in the warmed treatments T and TD. No correlations were observed between the functional diversity of the three leaf traits examined and annual biomass in the TDCO<sub>2</sub> treatment. Furthermore, measures of species diversity showed no relationship with aboveground biomass in any climate treatment (data not shown).

**Table 3. Pearson correlations between plant biomass production, community-level leaf traits and functional diversity**

	C	T	TD	TDCO <sub>2</sub>
<b>Community-scale leaf traits</b>				
SLA	<b>0.43</b>	-0.08	<b>-0.45</b>	<b>-0.56</b>
LDMC	<b>0.44</b>	<b>-0.54</b>	<b>-0.52</b>	-0.31
LNC	0.01	0.31	-0.1	0.05
<b>Functional Diversity</b>				
FD <sub>SLA</sub>	0.31	-0.12	-0.39	0.04
FD <sub>LDMC</sub>	0.41	<b>-0.51</b>	<b>-0.48</b>	0
FD <sub>LNC</sub>	0.2	0.04	-0.23	-0.18

## 5- Discussion

### **5.1 Aboveground biomass production and climate change**

Plant biomass production is both an important ecosystem property and a significant indicator of environmental change (Wang *et al.* 2007). Throughout the study period, we found that annual aboveground biomass responded strongly to warming and combined climate change (2080 climate scenario; simultaneous application of warming, summer drought and elevated CO<sub>2</sub>). Data from the present study confirms that warming has a significant impact on aboveground productivity in cool environments (Rustad *et al.* 2001; Bloor *et al.* 2010), and indicates that in the short-term, positive warming effects on aboveground biomass outweigh indirect, negative effects resulting from warming-induced changes in evapotranspiration and soil water content. However, aboveground biomass responses to prolonged warming did not match short-term results, and effects of both warming and combined climate change resulted in a progressive decline in aboveground biomass production over time.

Progressively-negative effects of warming on biomass may arise if initial, warming-enhanced vegetative growth occurs at the expense of stored plant reserves, and plants are unable to recover and restore reserves at a sufficiently rapid rate (Arft *et al.* 1999). Root biomass data obtained in 2009 provides support for this idea since root and rhizome biomass was significantly lower in the warmed (T) compared with the control treatment (R. Pilon, personal communication). Depletion of plant reserves may be exacerbated by increased plant respiratory costs under warmed conditions (Ryan 1991), reduced stomatal conductance and photosynthetic rates in drier soils associated with air warming (De Boeck *et al.* 2008) or exports of N in clipped biomass over and above soil N inputs (Barnes *et al.* 2007). Thus, the large exports of N in clipped biomass from warmed treatments at the start of the study, in the absence of fertilization, may have led to a reduction in N in the plant-soil system and contributed to progressive biomass decreases. These losses of N from the plant-soil system may have been further enhanced by increased nitrous oxide emissions observed under warmed conditions in the same experiment (Cantarel *et al.* 2011). Of course, progressive changes in biomass may also be driven by changes in community structure (Dukes *et al.* 2005), and the lower abundance of grasses in the warmed treatment at the end of our experiment could have contributed to the drop in annual biomass observed. Surprisingly, the increased abundance of legumes (and hence biological N fixation) in response to warming did not appear to buffer warming-induced reductions in annual productivity.

Previous grassland studies have demonstrated negative effects of summer drought on primary productivity, particularly when combined with high temperatures (Ciais *et al.* 2005; Grime *et al.* 2008; De Boeck *et al.* 2010). Contrary to expectations, we generally found weak summer drought effects on annual biomass production. We suggest that the 20% reduction in summer rainfall projected for 2080 in our high-rainfall study site does not represent a severe ecosystem stress over and above the soil drying associated with air warming alone. Above-average summer rainfall experienced during the study period probably attenuated drought impacts on biomass production, and may explain the finding that summer rainfall reduction was not systematically associated with a decrease in soil moisture. Resistance to small reductions in summer rainfall may also have been enhanced by patterns of grass phenology; in our study system, peak biomass occurs around mid-June and flowering is complete in early July (Bloor *et al.* 2010). Consequently, the grassland vegetation tends to senesce in July and August, followed by regreening and a period of vegetative growth in autumn. Reduced plant water demand for much of the summer period may have helped mitigate the negative impact of drought on biomass production. However, it is notable that during the dry 2009 year, a significant reduction in both soil water content and aboveground production was induced by the 20% reduction in summer rainfall. This result illustrates the possible occurrence of thresholds for ecosystem responses to climate change factors.

Long-term field experiments have shown that elevated CO<sub>2</sub> may have contrasting effects on grassland productivity, ranging from stimulatory effects which decline over time (Luo *et al.* 2004; Schneider *et al.* 2004; Reich *et al.* 2006), to progressive CO<sub>2</sub>-fertilisation effects observed after 3-4 years of study (Teyssonneyre *et al.* 2002; Niklaus & Korner 2004; Kamman *et al.* 2005; Kardol *et al.* 2010). In the present study elevated CO<sub>2</sub> had consistently positive effects on aboveground biomass (ranging from 8 % to 27 % across years), although these CO<sub>2</sub>-induced biomass responses were only significant in 2008 and 2009. This magnitude of CO<sub>2</sub> response is consistent with values documented in previous grassland studies (reviewed by Korner 2000; Ainsworth & Long 2005), and appeared to have a compensatory effect against drought-induced decreases in biomass throughout the study. Our data support the idea that elevated CO<sub>2</sub> has a protective effect against water stress due to improved plant water relations (Morgan *et al.* 2004), and confirms the finding that CO<sub>2</sub>-induced PNL may be overcome under warmed conditions (Hovenden *et al.* 2008). Unlike some other studies, elevated CO<sub>2</sub> was not associated with a shift in species composition or abundance of legumes/ graminoids (Potvin and Vasseur 1997; Korner 2000; Niklaus and

Korner 2004), which may indicate modified plant C allocation patterns and differential above- and belowground biomass sensitivity to elevated CO<sub>2</sub> (Owensby *et al.* 1993; Potvin and Vasseur 1997). To date, few studies have coupled the long-term dynamics of above- and belowground processes under combined warming and elevated CO<sub>2</sub>, and further work is required in this area.

## 5.2 Species diversity, plant functional traits and climate change

Trait-based approaches have emerged as a promising tool for understanding plant community assembly (McGill *et al.* 2006), scaling up from plant organ to ecosystem functioning (Garnier *et al.* 2004), and indicating environmental change (Lavorel & Garnier 2002). In line with Suding *et al.* (2008), we predicted that community-level leaf traits and functional diversity would respond more rapidly to climate change drivers than species diversity. Our data clearly supports this hypothesis; the lack of response to climate treatments in species richness, diversity (Shannon Weaver) and equitability observed in our five-year study contrasts with significant treatment effects on community-level leaf traits and functional diversity throughout the experimental period. Our results also agree with the assertion that perennial grassland community structure shows little response to climate change in unproductive and/or low diversity communities (Grime *et al.* 2000; Niklaus *et al.* 2001). Part of the explanation for the inertia of species diversity in our study may lie in plant trait variation; we found that trait divergence (a proxy for functional diversity) increased for all leaf traits in all treatments over the course of the experiment. Such phenotypic plasticity could buffer the effects of climate change on species interactions, and enhance community stability (Owensby *et al.* 1993).

In the present study we found that LDMC increased in response to combined climate change, whereas both LNC and SLA decreased under elevated CO<sub>2</sub> in agreement with previous work (Grime 2001). Such trait responses, generally attributed to a leaf-level accumulation of non-structural carbohydrates under elevated CO<sub>2</sub> (Soussana *et al.*, 1996; Korner 2000), should have a cascading effect on ecosystem productivity via changes in community-scale resource-use efficiency and litter quality (Lavorel & Garnier 2002). Simultaneous application of warming, summer drought and elevated CO<sub>2</sub> also had significant impacts on functional diversity. Combined climate change was associated with an increase in FD<sub>LDMC</sub> but a decrease in FD<sub>LNC</sub>. Whereas decreases in FD<sub>LNC</sub> suggest convergence in

photosynthetic investment at a community-level, increases in  $FD_{LDMC}$  are consistent with a higher variability of LDMC within the plant community. Plant trait variation is thought to play a key role in ecosystem productivity and stability in response to environmental perturbations (Yachi & Loreau 1999; Loreau *et al.* 2001). Given the potential for LDMC to mediate changes in ecosystem productivity (Lavorel & Garnier 2002), climate-induced changes in  $FD_{LDMC}$  may have indirect effects on grassland resistance to extreme climatic disturbances.

Irrespective of climate treatment, we found that community-level leaf traits best explained variations in annual plant biomass during the study period. These results are in agreement with data from natural grasslands measured under ambient climatic conditions (Vile *et al.* 2006; Mokany *et al.* 2008), and support the Mass Ratio Hypothesis (Grime 1998) which proposes that the functional traits of dominant species in a community largely determine ecosystem properties. Interestingly, all of the significant relationships between either mean leaf trait values or leaf-trait diversity and annual biomass production were positive in the control treatment and negative in the elevated temperature treatments (T/TD/TDCO<sub>2</sub>). For mean trait values, this simply indicates that biomass production is associated with the dominance of species with higher or lower trait values respectively. In contrast, positive relationships between functional diversity and production are often attributed to complementarity effects (Tilman 1997; Loreau *et al.* 2001; Mokany *et al.* 2008; Klumpp & Soussana 2009). These results suggest that for our study system, complementarity effects may be less prevalent under warmed conditions. Additional ecosystem processes (decomposition rates, belowground biomass production) need to be studied with functional approaches to better explain and anticipate changes in ecosystem processes under future climate conditions.

### 5.3 Study limitations

In the present study, we examined long-term responses of an upland grassland ecosystem to a published climatic scenario using gradient techniques to achieve projected increases in air temperatures. All the results discussed above are linked to the comparison of a single upland and lowland site which differ in air temperature; whilst every effort was made to ensure minimal site differences in factors other than temperature, similar approaches using other sites are clearly needed to test the generality of our findings. Our additive experimental design provides valuable insights into the impacts of both warming and projected climatic



conditions on grassland structure and function, but cannot address the important question of possible interactions between climatic drivers. The few, long-term climate change experiments with fully-factorial designs published to date suggest that drivers such as warming, rainfall and CO<sub>2</sub> are not additive, although the occurrence and duration of these interactions may vary over time (Dukes *et al.*, 2005; Hovenden *et al.*, 2008). Finally, as with most global change field experiments, the abrupt application of experimental treatments may be criticised for lacking biological realism. Whilst it seems reasonable to suppose that abrupt changes in climate may result in greater ecosystem responses compared with progressive increases in warming or atmospheric [CO<sub>2</sub>], such artefacts remain to be tested.

## 6- Conclusions

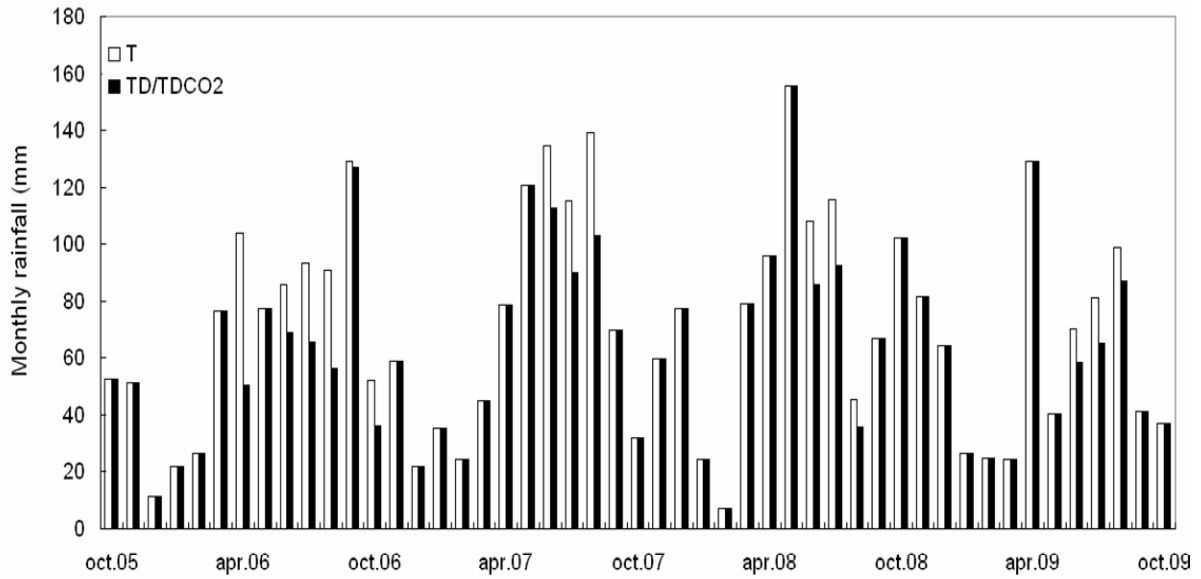
Overall we found that five-year exposure to projected climatic and atmospheric CO<sub>2</sub> conditions for 2080 had a significant negative effect on annual aboveground biomass production, driven primarily by ecosystem responses to warming. Stimulatory effects of elevated CO<sub>2</sub> on aboveground production were observed throughout the study period, but were insufficient to counterbalance the warming-induced reduction in productivity. Such a progressive, warming-induced decline in annual biomass production may result from a decline in plant reserves and a shift in the abundance of plant functional groups. Contrary to expectations, plant species diversity remained unaffected by climate change treatments even after five years. We suggest that resistance of species diversity to climate change may reflect increases in leaf trait variability and functional diversity, which serve to buffer the effects of climatic drivers. Future studies should address the role of phenotypic and genotypic variation for the sensitivity of grassland ecosystems to climate change.

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## 8- Appendix

*Appendix 1: Monthly averages of rainfall for the elevated temperature (T) and the combined temperature and summer drought (TD/TDCO<sub>2</sub>) treatments during the experimental period.*



## Résultats - Chapitre 2

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### **Impacts des facteurs du changement climatique sur la photosynthèse à l'échelle du couvert et à l'échelle foliaire, et réponses du métabolisme carboné chez les plantes en prairie permanente de moyenne montagne.**

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Les résultats du Chapitre 1 nous ont amené à nous interroger sur l'impact du changement climatique sur les processus physiologiques menant à la production aérienne. Ce chapitre s'intéresse donc aux échanges gazeux en CO<sub>2</sub> (flux-plantes-atmosphère) à l'échelle du couvert et à l'échelle foliaire (espèce dominante du couvert prairial : *Festuca arundinacea*) sous réchauffement et sous élévation de la concentration atmosphérique en CO<sub>2</sub>. Nous cherchons à caractériser la dynamique des échanges gazeux en CO<sub>2</sub> au travers de l'étude de l'allocation des ressources carbonées au sein d'une graminée (*F. arundinacea*). Nous espérons ainsi mieux comprendre les variations de biomasse observées sous réchauffement et sous élévation en CO<sub>2</sub>. Ce chapitre sera présenté sous forme d'un article en préparation s'intitulant 'Contrasting responses of photosynthesis and metabolism to warming and elevated CO<sub>2</sub> in an upland grassland'. Les co-auteurs sont Annette Morvan-Bertrand<sup>a</sup>, Marie-Pascale Prud'homme<sup>a</sup>, Marie-Laure Decau<sup>a</sup>, Marie Declos<sup>a</sup>, Jean-François Soussana<sup>c</sup> et Juliette Bloor<sup>b</sup>.

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a : UMR - Ecophysiologie Végétale, Agronomie & nutritons NCS Université de Caen, France

b : INRA - UR 874 Clermont-Ferrand, France

c : INRA - CODIR - ENVIRONNEMENT Paris, France

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## 1- Abstract

Cantarel et al. (Chapter 1) reported that warming has negative effects on annual aboveground biomass production of pasture monoliths after three years of climate change manipulation (*i.e.* in 2008). Moreover, annual aboveground biomass showed only limited positive responses to three years of atmospheric CO<sub>2</sub> enrichment. To better understand the effects i) of warming, ii) of elevated CO<sub>2</sub>, we studied both canopy-level CO<sub>2</sub> gas exchange and leaf-level CO<sub>2</sub> exchange for a dominant species (*Festuca arundinacea*) from May to September 2009. Moreover, we investigated carbon and nitrogen partitioning between leaf laminae and stubble for *F. arundinacea* via measurements of water-soluble carbohydrates, soluble leaf protein and C/N content. In line with our biomass findings, we expected a decrease in photosynthetic rates in warmed treatments compared to control treatments. Photosynthetic rates between ambient and elevated CO<sub>2</sub> treatments were expected to be similar, driven by a partial acclimation under elevated CO<sub>2</sub>. Canopy net assimilation rate was measured using a computer-controlled open-flow system and leaf gas exchange measurements were performed with a portable infrared gas analyser. Our results showed: (1) a negative effect of warming on canopy photosynthesis, leaf area index and canopy height; (2) a progressive acclimation of canopy photosynthesis to elevated CO<sub>2</sub> during the growing season; (3) effects of warming on *F. arundinacea* photosynthesis varied depending on measurement date, but were generally positive, whereas elevated CO<sub>2</sub> had no effect on leaf-level photosynthesis, suggesting complete acclimation; (4) for *F. arundinacea*, warming was associated with a decrease in leaf fructan content but an increase in leaf sucrose and fructose concentrations; (5) elevated CO<sub>2</sub> was associated with changes in *F. arundinacea* N balance (*i.e.* in leaf lamina and leaf stubble). Our results indicate that canopy-level responses to climate drivers are difficult to predict from leaf responses of individual species. Nevertheless, canopy-level data are consistent with climate-induced changes in aboveground biomass production. Our results suggest that longer-term decreases in canopy photosynthesis and production under warming may be driven by changes in canopy structure (decrease in canopy LAI and height), whereas effects of elevated CO<sub>2</sub> may be mediated by soil water content changes.



## 2- Introduction

Projected increases in atmospheric CO<sub>2</sub> concentration and global air temperatures for future decades are expected to have significant impacts on managed-land systems and ecosystem services (IPCC 2007). Given the potential for plant-atmosphere feedbacks via CO<sub>2</sub> exchange, the capacity of natural ecosystems to serve as sinks for atmospheric CO<sub>2</sub> under warming and elevated atmospheric CO<sub>2</sub> environments has become a major area of concern (Le Quere *et al.* 2009). Mechanistic understanding of the links between climatic factors and plant physiological responses at leaf- and vegetation stand-level is critical for predicting plant production in ecosystems under future climatic and atmospheric change (Körner 2003).

Both elevated CO<sub>2</sub> and warming can have significant impacts on plant physiology and growth. The effects of elevated CO<sub>2</sub> on leaf photosynthesis of grassland species are well-documented (Stirling *et al.* 1997; Rogers *et al.* 1998; Clark *et al.* 1999; Isopp *et al.* 2000). Typically, grassland species show an instantaneous increase in net CO<sub>2</sub> assimilation or photosynthesis (A) to an elevated atmospheric CO<sub>2</sub> concentration (Rogers *et al.* 1998). However, in leaves which are grown at elevated CO<sub>2</sub> concentrations for prolonged periods of time, CO<sub>2</sub>-induced increases in photosynthetic capacity are reduced, a phenomenon which has been termed as down regulation or acclimation (Long & Drake 1991). The photosynthetic acclimation could be due to both direct CO<sub>2</sub> control on photosynthesis and/or indirect CO<sub>2</sub> effects on plant N nutritional status (reviewed by Stitt & Krapp 1999). Thus acclimation is most pronounced in plants grown under low nutrient supply (Sage 1994), and nitrogen concentrations of plant tissues may be an important driver of leaf photosynthesis down regulation (Casella & Soussana, 1997). However, leaf level measurements alone are not sufficient to predict the CO<sub>2</sub> response of an ecosystem (Aeschlimann *et al.* 2005). The magnitude and direction of photosynthetic responses to elevated CO<sub>2</sub> may depend on other factors besides the morphological characteristics of the individual species, such as soil nutrient and water availability (Arp & Drake 1991) and / or degree of competition from other species (Bazzaz 1990).

Warming is well-known to be a driver of climate-induced changes for plant physiology and production (Rustad *et al.* 2001). In most studied species, elevated air temperatures increase rates of photosynthesis as long as the plants' optimal temperatures are

not exceeded (Berry & Björkman, 1980). Many plants show considerable phenotypic plasticity in their photosynthetic characteristics in response to warming, and plants grown at higher temperature generally have a higher optimal temperature of photosynthetic rate (Berry & Björkman, 1980). It is apparent that plants from habitats with large temperature variations during the growing season tend to possess a greater potential for acclimation over a wide temperature range (Berry & Björkman 1980). However, the degree of photosynthesis response to warming is species-specific and, just as with elevated CO<sub>2</sub>, the consequences for plant growth vary depending on resource availability and water stress (Berry & Björkman 1980; Badger *et al.* 1982; Makino *et al.* 1994; Yamasaki *et al.* 2002). Warming may affect the photosynthetic rates of plants indirectly via changes in evapotranspiration rates and soil moisture status (Rustad *et al.* 2001) or modified rates of litter and soil organic matter decomposition and nutrient mineralization (Jonasson *et al.* 1999).

Responses of photosynthesis to elevated CO<sub>2</sub> and warming depend in part on the plant's capacity to adjust to these two environmental factors via changes in photosynthetic enzymes and proteins or water-soluble carbohydrates (Berry & Björkman 1980; Drake *et al.* 1996). Water-soluble carbohydrates are both the product of photosynthesis and the substrate for sink metabolism i.e. leaves and root growth zones (Rogers & Ainsworth 2006). Accumulation of carbohydrates is one of the most observed changes in leaves of plants grown at elevated CO<sub>2</sub> (Farrar & Williams 1991). The most pronounced increases are in the levels of sucrose and the transient storage polysaccharides such as fructan (Long & Drake 1991). Accumulation of carbohydrates in leaves may result from an insufficient sink demand (Stitt 1991; Krapp *et al.* 1993; Webber *et al.* 1994) or a reallocation of N away from RubisCO to other N-pools within the plant (Xu *et al.* 1994). Plant-level source-sink relationships appear to play a crucial role in determining the size and direction of photosynthetic response to elevated CO<sub>2</sub>. Indeed, accumulation of soluble carbohydrates in source leaves has been associated with the repression of photosynthetic gene expression, thus providing a mechanistic link between sink limitation and photosynthetic acclimation (Sheen 1990; Krapp *et al.* 1993; Van Oosten & Besford 1994).

The present study aims at providing insight into mechanisms that drive canopy response to climate and atmospheric change for grasslands exposed to air warming and elevated CO<sub>2</sub>. Previous work (Cantarel *et al.*, unpublished data and see Chapter 1) has shown a decrease in grassland biomass production for experimental monoliths after three years of



warming. In the same climate change study, Cantarel and coworkers found limited biomass responses to elevated CO<sub>2</sub>. We hypothesize that these patterns of biomass production may reflect acclimation in plant photosynthetic rates. In line with biomass results, we predict a decrease of photosynthetic rates in warmed treatments compared to unwarmed control conditions but similar photosynthetic rates between ambient and elevated CO<sub>2</sub> treatments. We examine how photosynthesis responds to warming and elevated CO<sub>2</sub> at both the plant and community-level over the course of a growing season. We investigate if potential acclimation of the grassland canopy to elevated CO<sub>2</sub> can be explained by greater accumulation of sugars during the growing season under elevated CO<sub>2</sub>. Canopy and leaf CO<sub>2</sub> gas exchange was followed from May to September 2009, along with leaf water-soluble carbohydrates, soluble leaf protein and C/N content to analyse plant responses to warming and elevated CO<sub>2</sub>.

### **3- Materials and methods**

#### ***3.1 Site description and experimental design***

The Clermont Climate Change Experiment is located in French Massif Central region (45°47'N, 03°05'E). The study site is an upland permanent grassland, characterised by a Cambisol soil (59.5 % sand, 19.7 % silt, 20.8 % clay, pH<sub>H2O</sub> 6.2). The plant community is dominated by grasses (*Festuca arundinacea*, *Arrhenaterrum elatius*, *Elytrigia repens*, *Poa pratensis*). This long-term field experiment was initiated in May 2005, manipulating air temperature, summer rainfall and atmospheric CO<sub>2</sub> in an additive, replicated design in line with IPCC projections for the Massif Central region in 2080 (ACACIA A2 scenario, IPCC 2001). The experiment consists of 80 grassland monoliths (0.5 x 0.5 x 0.4 m in size) extracted from an extensively-managed upland semi-natural grassland site (Theix, 45°43'N, 03°01'E, 850 m a.s.l.) and allocated at random to one of four climate treatments; C (control), T (+ 3.5 °C), TD (+ 3.5 °C, 20 % reduction in summer rainfall) and TDCO<sub>2</sub> (+ 3.5 °C, 20 % reduction in summer rainfall, CO<sub>2</sub> level of 600 ppm). Four monoliths are grouped together to form an experimental unit (five experimental units per climate treatment, for full details see Bloor *et al* 2010).

Monoliths allocated to the Control treatment (C) remain at the upland site “Theix”, under ambient weather conditions (8.7 °C for mean annual temperature and 780 mm for mean

annual precipitations). Elevated temperatures were achieved by transporting monoliths to a lower-altitude site 14 km away (Clermont-Ferrand, 45°47'N, 03°05'E, 350 m a.s.l.). The lowland site is characterised by a mean annual temperature of 11.1 °C and a mean annual rainfall of 374 mm (38 year averages). Given consistently lower monthly rainfall levels at the lowland site, rainfall in the T treatment is supplemented by irrigation to generate similar rainfall patterns to the C treatment in upland site. Summer drought in the TD and TDCO<sub>2</sub> treatment is obtained by a reduction of irrigation during June, July and August. At all other times differences in precipitation between C and TD, TDCO<sub>2</sub> are minimized by supplemental watering. Mini-FACE (Free Air Carbon dioxide Enrichment) technology is used to establish the elevated CO<sub>2</sub> treatment (~ 600 ppm); the target CO<sub>2</sub> concentration was operational during daylight hours and is maintained via input/output signals at an automated control system.

Climate measurement are made at both the upland and the lowland sites using a Campbell Scientific automatic weather station and logged to a CRX-10 data logger (Campbell scientific Inc., Utah, USA) at 30 minute intervals. Potential evapotranspiration of vegetation (ETP) were hourly calculated using FAO-Penman-Montheis equation (see FAO, 2005) for C, T and TD treatments. The ETP equation was adjusted with a coefficient developed by Olioso *et al.* (2010) for the TDCO<sub>2</sub> treatment considering possible CO<sub>2</sub> effects on ETP. Volumetric soil moisture (0-20 cm) is recorded hourly in each climate treatment using ECH<sub>2</sub>O-20 probes (Dielectric Aquameter, Decagon Devices, Inc., Pullman, WA; n = 3). Since April 2005, all grass monoliths in the different climate treatments have been cut to a height of 5 cm at six-month intervals (April and October) and no fertiliser has been applied, in line with management practices at the upland site. In 2009, cuts occurred the 23<sup>rd</sup> April and the 23<sup>rd</sup> October.

### **3.2 Photosynthetic Gas exchange**

For this study, canopy and leaf photosynthetic measurements were carried out from May to September 2009, at 3-week intervals, to assess the effects of, warming and elevated CO<sub>2</sub> on photosynthesis during the growing season of the plant community. Two measurement units were built to measure simultaneously C and T or TD and TDCO<sub>2</sub> treatments, each measurement unit consisting of two chambers which analysed two monoliths from the same treatment (Figure 1). These measurements were carried out continuously for one day (24 hours). Canopy photosynthetic measurements were made on four replicates of each treatment

over four days with similar climate conditions; two days for four replications of the C-T treatment pair and two additional days for the TD-TDCO<sub>2</sub> treatment pair.

Canopy net assimilation rate was measured using a computer-controlled open-flow system (Figure 1). Prior to the start of the experiment, a permanent stainless steel base was inserted around each target monolith (14 cm high, inserted 10 cm in the soil); the upper rim had a rubber gasket to ensure a tight seal. Monolith vegetation was placed in a transparent plastic chamber (Lexan ®, 1 mm thickness, volume: 0.5 x 0.5 x 0.5, approximately 150 L). Light inside the chambers did not differ significantly from ambient light conditions (Student test  $P > 0.05$ , data not shown). The air (sampled at 3m height to avoid Mini-FACE CO<sub>2</sub> contamination) was circulated in the canopy enclosure by a fan unit (400 to 600 L min<sup>-1</sup> ~ 3 to 4 volumes per minute). In order to increase the precision of the night respiration measurements, the flow rate was decreased automatically to 150 L min<sup>-1</sup> between 21:30 h and 06:30 h by adjusting the air flow. For the CO<sub>2</sub> fumigated treatments, the air flow was enriched with CO<sub>2</sub> from the same bulk cylinder as the MiniFACE system and managed by a mass flow controller (Tylan SC2900, Tylan General, Torrance, CA, USA) driven by the laptop. The flow rate through the enclosure was determined using two hotwire flow meters (TSI 8450, TSI GmbH, Aix La Chapelle, Germany). Inlet and outlet CO<sub>2</sub> concentrations were measured using a differential infra-red CO<sub>2</sub> analyser (Rosemount Binos 100, Emerson Electric Co., St Louis, MO, USA) calibrated before each measurement campaign with a 408 ppm [CO<sub>2</sub>] CO<sub>2</sub> standard (Messer-Griesheim, Germany). The mean air temperature inside the chambers was slightly higher during chamber measurements compared with outside air temperatures (+ 0.9 ± 0.6 °C during day and +1.3 ± 0.5 °C at night).

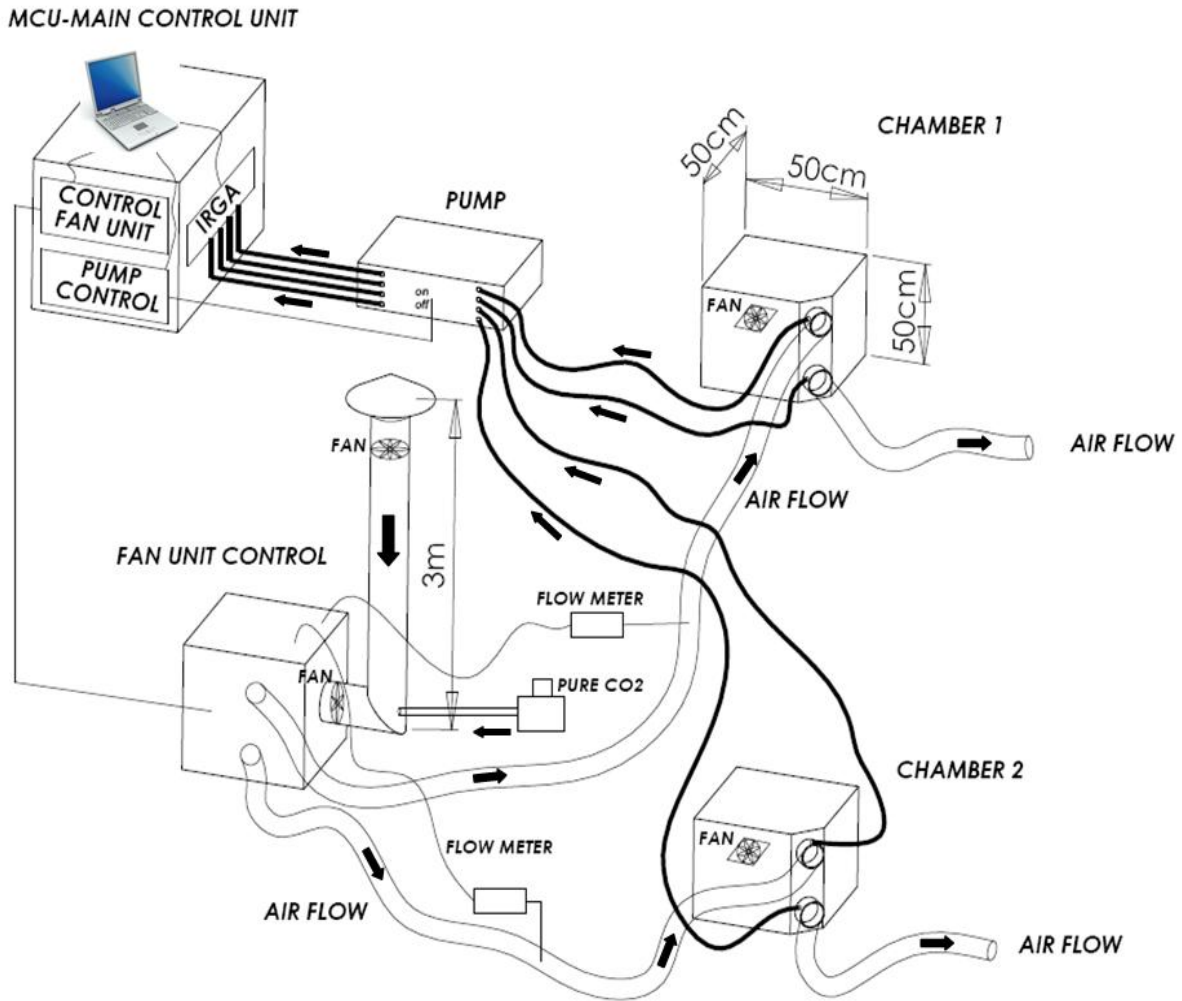


Figure 1: Schematic diagram of an automated open-flow gas exchange device used to measure canopy  $\text{CO}_2$  exchange rates. The  $\text{CO}_2$  concentration was measured sequentially with an IRGA at four points in the air system: at the inlet and the difference between inlet and outlet of chamber 1 and at the inlet and the difference between inlet and outlet of chamber 1.

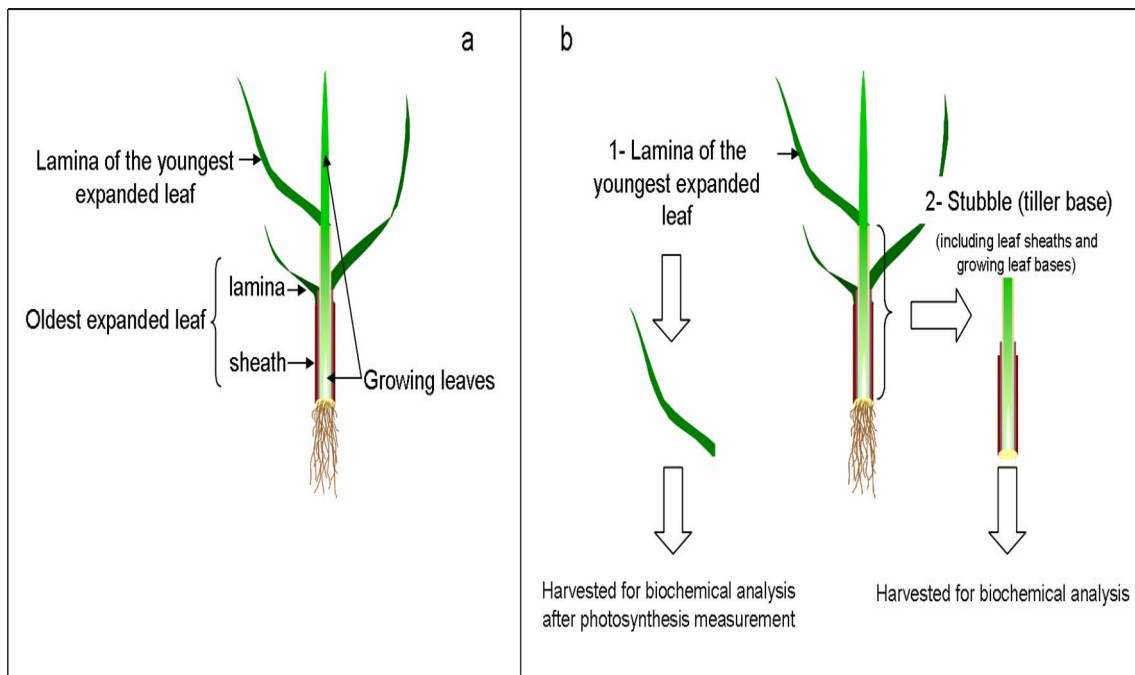
The response of canopy net assimilation rate ( $A$ ,  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ) to transmitted radiation (PAR) was fitted to a simple rectangular hyperbola following Ruimy *et al.* (1995):

$$A = \{(A_{\max} \cdot \alpha \text{PAR}) / (K_m + \alpha \text{PAR})\} - R_{\text{eco}}$$

where  $A_{\max}$  ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), is the maximal photosynthetic rate of the canopy;  $K_m$  ( $\mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ ), is the transmitted PAR needed to obtain half  $A_{\max}$ ;  $\alpha$  ( $\mu\text{mol CO}_2 \mu\text{mol PAR}^{-1}$ ), apparent quantum efficiency; and  $R_{\text{eco}}$  ( $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$ ), is the ecosystem dark respiration rate. Only, canopy-level photosynthetic rates per unit ground area standardised at 1000  $\mu\text{mol PPF}$  (Photosynthetic Photon Flux Density) ( $A_{1000}$ ),  $\alpha$  and  $K_m$  are reported in this study. The ecosystem light compensation point (LCP;  $\mu\text{mol PAR m}^{-2} \text{ s}^{-1}$ ) was calculated as  $\text{LCP} = (-R_{\text{eco}} \cdot K_m) / (A_{\max} - R_{\text{eco}})$ . After each canopy  $\text{CO}_2$  exchange measurement, leaf index area (LAI),

fraction of absorbed photosynthetically active radiation (% faPAR) and height (cm) of grass vegetation were measured. LAI and faPAR was measured using a sunfleck ceptometer (Decagon Devices Inc., Pullman, WA, USA) placed successively above and below (at the ground level) the canopy of the monoliths. Radiation use efficiency (RUE; mol CO<sub>2</sub> mol photon<sup>-1</sup>) were calculated with faPAR and A<sub>1000</sub> values for each date, as RUE = A<sub>1000</sub> / (1000 faPAR).

Leaf gas exchange measurements were performed with a portable infrared gas analyser system (LiCOR 6400, LiCOR Inc., Lincoln NE, USA). Photosynthetic capacity under non-limiting light conditions (photon flux density, PPFD, of 1000 μmol m<sup>-2</sup> s<sup>-1</sup>) or LeafA<sub>1000</sub> was measured on leaf laminae of a dominant grass species (*F. arundinacea*) under controlled temperature conditions (leaf temperature between 20 and 25 °C) in the chamber. The C and T comparison was made at a fixed CO<sub>2</sub> concentration of 380 ppm (ambient conditions). Leaf photosynthetic measurements in the TD treatment were performed at 380 and 600 ppm, whereas leaf photosynthetic measurements in the TDCO<sub>2</sub> were at 600 ppm only. These measurements aim to compare short term (TD at 600 ppm) and long term (TDCO<sub>2</sub>) responses to an increase in atmospheric CO<sub>2</sub> concentrations. This comparison therefore allows to determine the degree of photosynthetic acclimation of plants grown under elevated CO<sub>2</sub> (TDCO<sub>2</sub>). Three replicate laminae were recorded for each climate treatment and measurement date. All measurements were made on vegetative individuals, and the lamina measurement was made from the first fully expanded leaf (Figure 2). After photosynthetic measurements, measured individual tillers were collected and placed on ice. Measured lamina and stubble (see Figure 2 for full details) were reserved. A fresh lamina subsample (~ 50 mg) was frozen with liquid nitrogen for soluble protein extraction and stored at -80 °C. Remainder of measured lamina and the associated stubble were freeze-dried for water-soluble carbohydrate extraction (WSC). In August, leaf photosynthetic measurements of *F. arundinacea* were not sampled for physiological analyses because of very dry conditions. The leaf lamina area enclosed by the analyser chamber was traced onto a sheet and its area analysed using an electronic planimeter (LI 3100, Li-Cor, Lincoln, NE, USA).



**Figure 2: Schematic views of (a) a *F. arundinacea* tiller and (b) samples harvested for biochemical analysis (1- leaf lamina and 2- stubble)**

### 3.3 Extraction and measurements of water soluble carbohydrate (WSC)

Fifty milligrams of freeze-dried plant tissue (lamina and stubble), ground to a fine powder, were placed in a 14 mL polypropylene round-bottom tube (Falcon Becton Dickinson Labware, Franklin Lakes, NJ, USA) with 2 mL of 80 % ethanol (v/v) containing Mannitol at 0.5g/L. The tube contents were mixed and incubated for 20 min at 80°C. After ethanol extraction, the sample was centrifuged at 10000g for 10 min (LYOVAC GT2, STERIS, USA). The supernatant was preserved and 2 mL of water was added to the pellet. The tube contents were mixed and incubated 20 min at 60 °C. After the first aqueous extraction, the sample was centrifuged (Avanti J-E, Beckman-Coulter, CA, USA) at 10000 g for 10 min and the operation was repeated one time. The supernatant was preserved. The pellet was freeze-dried for insoluble C/N analyses. The three supernatants were pooled, evaporated to dryness under vacuum (Speedvac concentrator 5301, Eppendorf, France) and the residue was dissolved in 1.5 mL water. A subsample of carbohydrate extract was reserved for soluble C/N analyses. Aliquots of carbohydrate extract (100 µL) were passed through minicolumns (Mobicols from MoBITec, Göttingen, Germany) packed, from bottom to top, with 150 µL Amberlite CG-400 II, Cl<sup>-</sup> form (Fluka, Buchs, Switzerland), 80 µL polyvinylpyrrolidone (Sigma-Aldrich, St. Louis, USA), and 250 µL of Dowex 50W X8-400 H<sup>+</sup>-form (Sigma-Aldrich) to remove pigments and charged compounds. Glucose, fructose, sucrose and fructans

were quantified by high-performance liquid chromatography (HPLC) on a cation exchange column (Sugar-PAK, 300 m x 6.5mm, Millipore Waters, Milford, MA USA) elude with 0.1 mM CaEDTA in water, using mannitol as an internal standard (Guerrand *et al.* 1996) and refractometer as a sugar detector.

### ***3.4 Determination of soluble and insoluble N and C***

Aliquots of WSC extract freeze-dried pellets (1.5 µg) were placed into tin capsules (Europa Scientific Ltd, UK) for insoluble carbon and nitrogen analysis. For liquid aliquots of WSC extract (100 µL), Chromosorb ® (Europa Scientific Ltd, Crewe, UK) were added into tin capsules and dried at 40°C during 24 hours for soluble carbon and nitrogen analysis. The total N and C in soluble and insoluble fractions were determined with a continuous flow isotope mass spectrometer (Isoprime, GV Instrument, Manchester, UK) linked to a C/N/S analyser (EA 3000, Euro Vector, Milan, Italy). In brief, C compounds in soluble fraction are mainly water-soluble carbohydrates, organic acids, amino acids and amides. N compounds in soluble fraction are mainly nitrate, ammonium, amino acids and amides. Soluble proteins were denatured during extraction by high temperature. C compounds in insoluble fraction are mainly starch, lipids and cell-wall compounds. N compounds in insoluble fraction are mainly denatured soluble proteins, cell-wall and membrane proteins.

### ***3.5 Extraction and measurements of total leaf soluble proteins***

The proteins (soluble proteins and RubisCO) were extracted from frozen leaf subsamples (50 mg), ground to a fine powder with 20 mg polyvinylpyrrolidone (Sigma-Aldrich, St. Louis, USA) and mixed with 200 µL of MacIlvaine (pH 6.8; Citrate – Phosphate Buffer). This was followed by centrifugation at 12000 g at a temperature of 4°C for 1h (Avanti J-E, Beckman-Coulter, CA, USA). The supernatant was centrifuged at 12000 g for 5 min. The total protein concentration was measured in a solution of supernatant and 200 µL Bradford reactive (Bio-Rad Laboratories, Hercules, CA, USA; for more details see Bradford 1976).

### ***3.6 Statistical analysis***

Due to the additive design of the experiment, effects of warming were determined by comparing the C and T treatments; effects of elevated [CO<sub>2</sub>] by comparing TD and TDCO<sub>2</sub>.

Effects of climate treatment on canopy and leaf photosynthesis, sugars and proteins were analysed using mixed model repeated measures analysis of variance (ANOVA) with both treatment and date as fixed factors (Zar 1998). Climate effects for individual measurement dates were analyzed using one-way ANOVA. When necessary, data were transformed prior to analysis to conform to assumptions of normality and homogeneity of variance. All statistical analysis was performed using the Statgraphics Plus 4.1 (Statistical Graphics Corp., Rockville, Maryland, USA) statistical package.

## **4- Results**

### ***4.1 Meteorological conditions and climate treatments***

From May 2009 to September 2009, the mean difference in monthly temperatures between the control (C) and warmed (T) treatments was  $3.4 \pm 0.2$  °C and the cumulated difference in precipitation was  $20.9 \pm 0.3$  mm (i.e. less than 4 % of the total rainfall during this period) (see chapter 1). Soil water status in the T treatment was only lower than in the C treatment in May and September (Appendix 1a). Nevertheless, water stress for plants grown in the warmed treatment was higher compared to plants grown in control treatment (cumulative rainfall divided by cumulative potential ETP for C and T treatments, Appendix 2). In summer 2009 (June, July, August), the droughted (TD, TDCO<sub>2</sub>) treatments received a 21 % reduction in rainfall compared with the warmed only (T) treatment. The mean daily atmospheric CO<sub>2</sub> concentration difference between TDCO<sub>2</sub> and TD reached  $198.5 \pm 8.7$  ppm. Moreover, there was no significant difference in atmospheric CO<sub>2</sub> concentration across ambient CO<sub>2</sub> treatments (C, T and TD). Soil moisture in TDCO<sub>2</sub> treatment was significantly higher than in the TD treatment in July, August and September (Appendix 1b).

During the canopy gas exchange measurements, there was no significant difference in soil moisture between C and T (Table 1). However, the air temperature inside the measurement chambers was  $2.5 \pm 0.8$  °C and  $4.0 \pm 0.7$  °C higher in T compared to C for day and night temperatures, respectively. There was no significant difference in day and night temperature inside the canopy enclosures between TD and TDCO<sub>2</sub> (Table 1). Moreover, CO<sub>2</sub> concentrations showed no significant difference inside and outside the chambers during canopy measurements (data not shown). In July and August, soil moisture was significantly



higher in TDCO<sub>2</sub> compared with TD, but no treatment differences were observed for soil moisture in measurement dates during May and June (Table 1). Over the study period, PAR values ranged between 0 and 1600  $\mu\text{mol PPF}$  and the daily PAR was 720  $\mu\text{mol PPF}$  on average across all climate treatments.

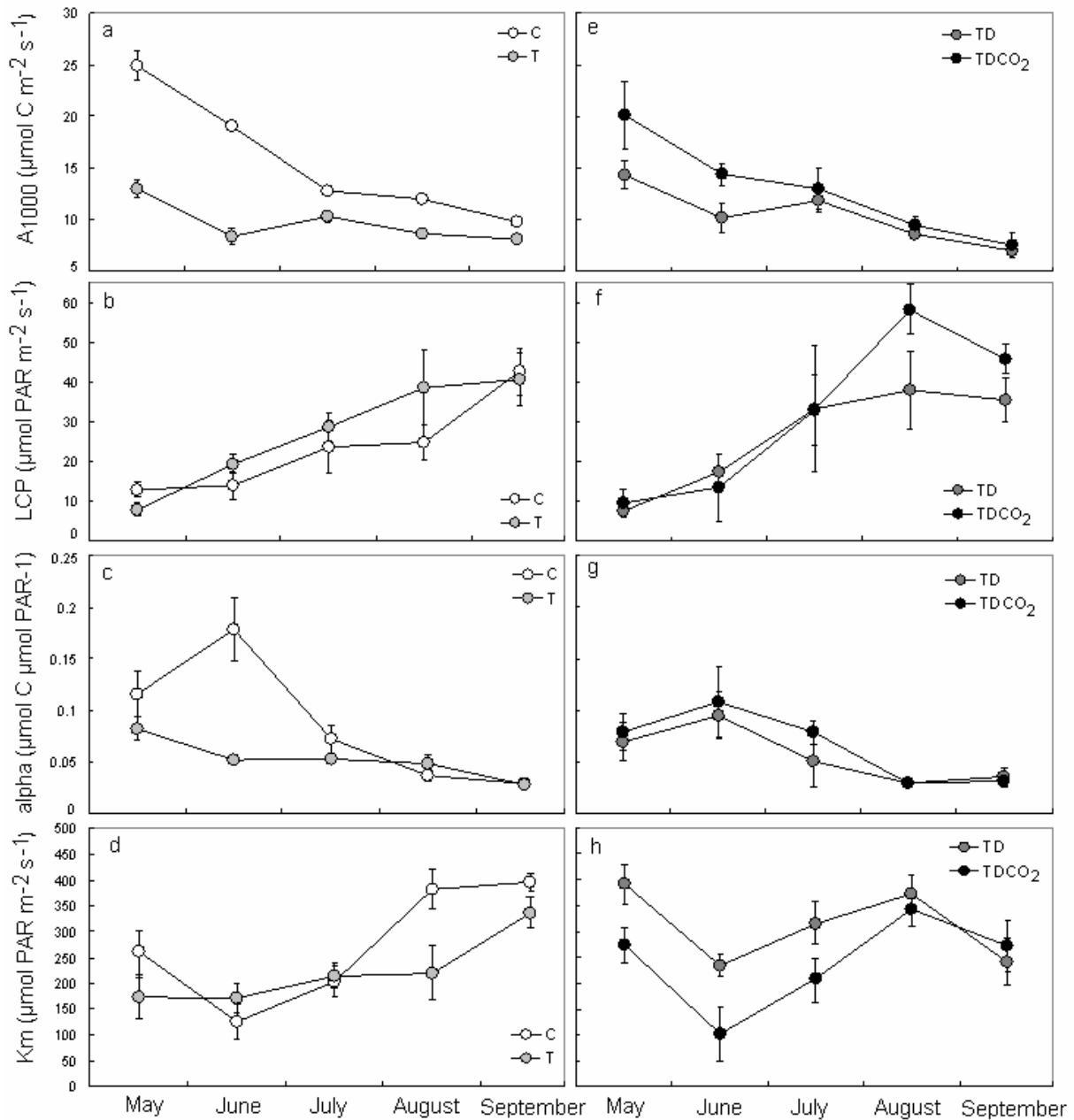
**Table 1: Mean soil moisture (soil water content, %) and air temperature (night and day) recorded during each measurement date of CO<sub>2</sub> canopy exchange for warming (a) and elevated CO<sub>2</sub> (b) treatments. Means and standard errors are presented (n = 15).**

		May	June	July	August	September
<b>a) Warming impact</b>						
Soil water content (%)	C	8.7 ± 1.2	10.3 ± 0.8	4.6 ± 0.7	4.4 ± 0.2	6.1 ± 0.3
	T	7.6 ± 0.3	10.1 ± 0.6	4.7 ± 0.6	4.1 ± 0.7	5.6 ± 1.3
Diurnal temperature (°C)	C	18.1 ± 2.2	16.9 ± 0.6	22.7 ± 1.7	24.9 ± 3.1	22.8 ± 0.6
	T	22.3 ± 2.1	22.2 ± 1.4	24.6 ± 1.9	29.5 ± 0.8	22.3 ± 0.6
Night temperature (°C)	C	7.9 ± 2.2	8.3 ± 1.4	7.3 ± 0.8	13.3 ± 1.7	8.4 ± 2.3
	T	12.6 ± 2.1	12.3 ± 1.8	12.6 ± 0.4	16.2 ± 0.3	11.9 ± 0.5
<b>b) Elevated [CO<sub>2</sub>] impact</b>						
Soil water content (%)	TD	7.2 ± 0.5	4.6 ± 0.5	4.9 ± 0.7	3.1 ± 1.1	5.7 ± 0.7
	TDCO <sub>2</sub>	6.8 ± 0.3	6.6 ± 1.1	8.6 ± 0.8	5.3 ± 2.3	5.5 ± 0.2
Diurnal temperature (°C)	TD	22.1 ± 0.5	23.6 ± 0.7	23.2 ± 1.2	30.7 ± 1.1	18.7 ± 0.7
	TDCO <sub>2</sub>	21.9 ± 0.6	23.3 ± 0.7	23.2 ± 0.9	30.9 ± 0.5	18.1 ± 0.5
Night temperature (°C)	TD	13.3 ± 1.5	12.1 ± 1.4	11.4 ± 1.5	17.6 ± 1.1	10.1 ± 0.7
	TDCO <sub>2</sub>	13.3 ± 1.5	12.1 ± 1.6	11.2 ± 1.6	17.4 ± 1.1	10.1 ± 0.6

#### 4.2 Effects of warming and elevated CO<sub>2</sub> on canopy gas exchange

Canopy-level photosynthetic parameters showed significant variation across measurement dates (Figure 3). During the study period, A<sub>1000</sub> ranged between 6 and 27  $\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$  across treatments. A<sub>1000</sub> was significantly affected by warming and by measurement date (repeated measures ANOVA), but warming effects varied over time (significant Treatment x Date interaction;  $F_{1,4} = 26.9$ ,  $P < 0.001$ , Figure 3a). Warming had a negative impact on A<sub>1000</sub> values across measurement dates, but differences in A<sub>1000</sub> between C and T decreased over time (Fig 3a). Apparent quantum efficiency ( $\alpha$ ) and Km also showed a significant Treatment x Date interaction ( $F_{1,4} = 10.6$ ,  $P < 0.001$  and  $F_{1,4} = 14.6$ ,  $P < 0.001$  respectively, Figure 3c, 3d). Patterns of response of  $\alpha$  were driven by negative effects of warming in June ( $F_{1,6} = 22.5$ ,  $P < 0.01$ ) whereas Km showed significant negative effects of warming in August and September ( $F_{1,6} = 8.4$ ,  $P < 0.05$ ;  $F_{1,6} = 6.8$ ,  $P < 0.05$ ). In contrast, light compensation point (LCP) showed

no response to warming; instead LCP showed a significant response to date ( $F_{1,4} = 15.9$ ,  $P < 0.001$ , Figure 3b) with a constant increase over the course of the growing season.



**Figure 3: Effects of warming (a,b,c,d) and elevated CO<sub>2</sub> (e,f,g,h) on photosynthetic rate at 1000 μmol of PAR (A<sub>1000</sub>; a, e), ecosystem light compensation point (LCP; b, f), apparent quantum efficiency (α; c, g) and transmitted PAR needed to obtain half A<sub>max</sub> (K<sub>m</sub>; d, h). Treatments are given by: C = Control; T = elevated temperature (+3.5°C); TD = elevated temperature and summer drought (+ 3.5 °C, - 20 %); TDCO<sub>2</sub> = elevated temperature, summer drought and CO<sub>2</sub> enrichment (+ 3.5 °C, - 20 %, + 200 ppm). Means and standard errors are presented per treatment and measurement date (n = 4).**

Elevated CO<sub>2</sub> had a significant effect on A<sub>1000</sub> but effects of elevated CO<sub>2</sub> varied depending on date (significant Treatment x Date interaction,  $F_{1,4} = 4.61$ ,  $P < 0.05$ , Figure 3e).

Elevated CO<sub>2</sub> had a positive effect on A<sub>1000</sub> rates in May and June (F<sub>1,6</sub> = 7.5, P < 0.05; F<sub>1,6</sub> = 6.8, P < 0.05 respectively) but no effect from July onwards. LCP showed a tendency to increase in response to elevated CO<sub>2</sub> (F<sub>1,2</sub> = 3.4, P < 0.1, Figure 3f), which was driven by August and September measurements. LCP also showed significant effects of date (F<sub>1,2</sub> = 14.8, P < 0.001) with higher values in August.  $\alpha$  showed significant effects of date only (F<sub>1,4</sub> = 7.4, P < 0.001, Figure 3g) with lower  $\alpha$  values in August and September compared to the other dates. Km showed a negative effect of elevated CO<sub>2</sub> (F<sub>1,2</sub> = 6.5, P < 0.05, Figure 2h), which was consistent between dates (no significant Treatment x Date interaction). In addition, Km varied with date, having lower values in June (F<sub>1,4</sub> = 5.7, P < 0.01).

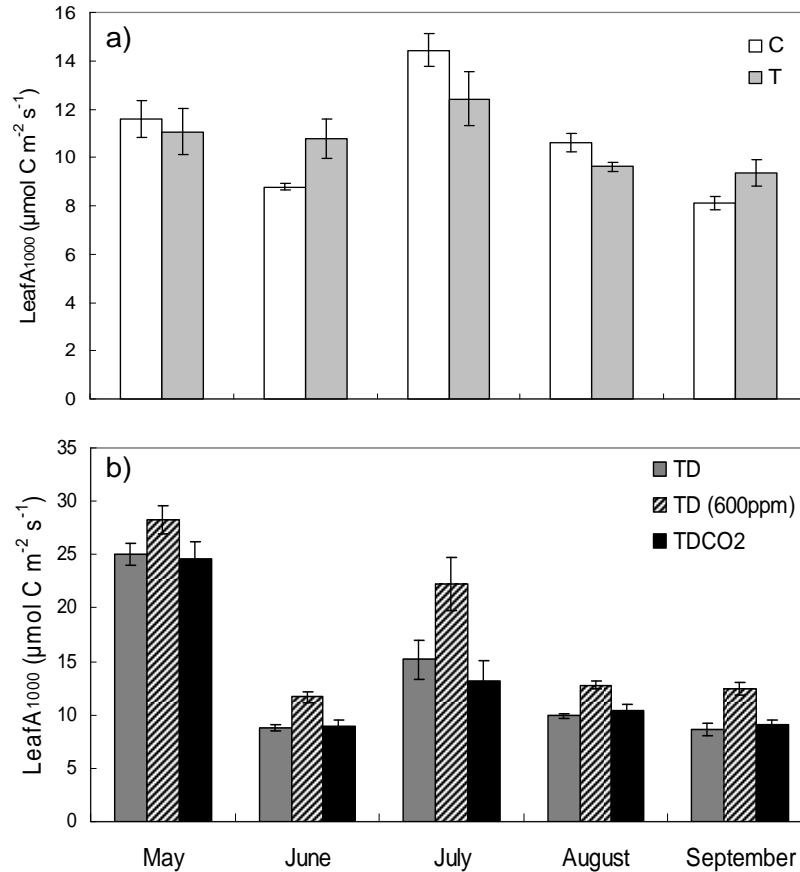
#### ***4.3 Impact of warming and elevated CO<sub>2</sub> on canopy height, canopy Leaf Area Index (LAI), Radiation Use Efficiency (RUE) and fraction of absorbed Photosynthetically Active Radiation (faPAR)***

Across all climate treatments and dates, canopy height ranged from 15 to 40 cm, LAI values ranged from 0.4 to 5.8, and RUE values from 0.008 to 0.05 mol CO<sub>2</sub> mol photon<sup>-1</sup>. Overall, warming had a negative effect on canopy height, LAI and faPAR (Table 2), and effects of warming did not differ between dates (no significant Treatment x Date interaction). Unlike warming, elevated CO<sub>2</sub> had no effect on canopy height during the experiment. Nevertheless, elevated CO<sub>2</sub> had a positive effect on LAI and faPAR (Table 2). Irrespective of climate treatment, RUE values decreased over the course of the growing season. Effects of warming on RUE varied depending on measurement date (significant Date x Treatment interaction, Table 2); warming had a positive impact in July. In contrast, elevated CO<sub>2</sub> had no effect on RUE during the study.

#### ***4.4 Effects of warming and elevated CO<sub>2</sub> on leaf photosynthesis in Festuca arundinacea***

Over the course of the experiment, leaf-level photosynthetic rates recorded for *F.arundinacea* ranged from 7 – 36  $\mu\text{mol C m}^{-2} \text{s}^{-1}$  across all climate treatments. Warming effects on A<sub>1000</sub> varied over time (significant Treatment x Date interaction, F<sub>1,4</sub> = 7.3, P < 0.001, Figure 3). In June and September, A<sub>1000</sub> values increased under warming (F<sub>1,6</sub> = 6.69, P < 0.01 and F<sub>1,6</sub> = 14.7, P < 0.001 respectively), whereas in August, warming had a negative effect on A<sub>1000</sub> values (F<sub>1,6</sub> = 18.9, P < 0.001). Irrespective of measurement date, TD leaves at 600 ppm showed a significant increase in A<sub>1000</sub> compared with TD leaves at 380 ppm.

However, no differences in LeafA<sub>1000</sub> were found between TD leaves recorded under ambient CO<sub>2</sub> conditions (380 ppm) and TDCO<sub>2</sub> leaves at 600 ppm CO<sub>2</sub> ( $F_{1,4} = 0.3$ ,  $P > 0.05$ ), suggesting an acclimation of LeafA<sub>1000</sub> rates in the TDCO<sub>2</sub> treatment.



**Figure 3: Effects of warming (a) and elevated CO<sub>2</sub> (b) on leaf net photosynthesis ( $A_{1000}$ ) of *Festuca arundinacea* from May to September 2009. Leaf  $A_{1000}$  values in C, T and TD treatments were measured at 380 ppm (ambient CO<sub>2</sub> conditions), whereas in TD600 and TDCO<sub>2</sub>, Leaf $A_{1000}$ , were measured at 600 ppm (elevated CO<sub>2</sub> conditions). Means and standard errors are presented per treatment and date ( $n = 12$ ).**

#### 4.5 Leaf-level water soluble carbohydrates in *Festuca arundinacea* under warming and elevated CO<sub>2</sub>

Across climate treatment and date, fructans accounted for  $72.2 \pm 1.5$  and  $83.6 \pm 1.3$  % of the water soluble sugars (WSC) in the leaf lamina and stubble, respectively (Table 3 and Table 4). The remaining WSC could be ranked: sucrose > fructose > glucose, in leaf lamina and stubble across treatments. Total WSC concentration was greater in stubble compared to the leaf lamina for all climate treatments. Fructans and glucose concentrations of leaves varied with time (Table 4). Fructans concentration increased in June and July, and glucose concentrations

decreased in July. Leaf glucose concentrations were stable both within dates and climate treatments. For leaf stubble, fructans concentration was lowest in May whereas sucrose concentrations were significantly higher in June (Table 4).

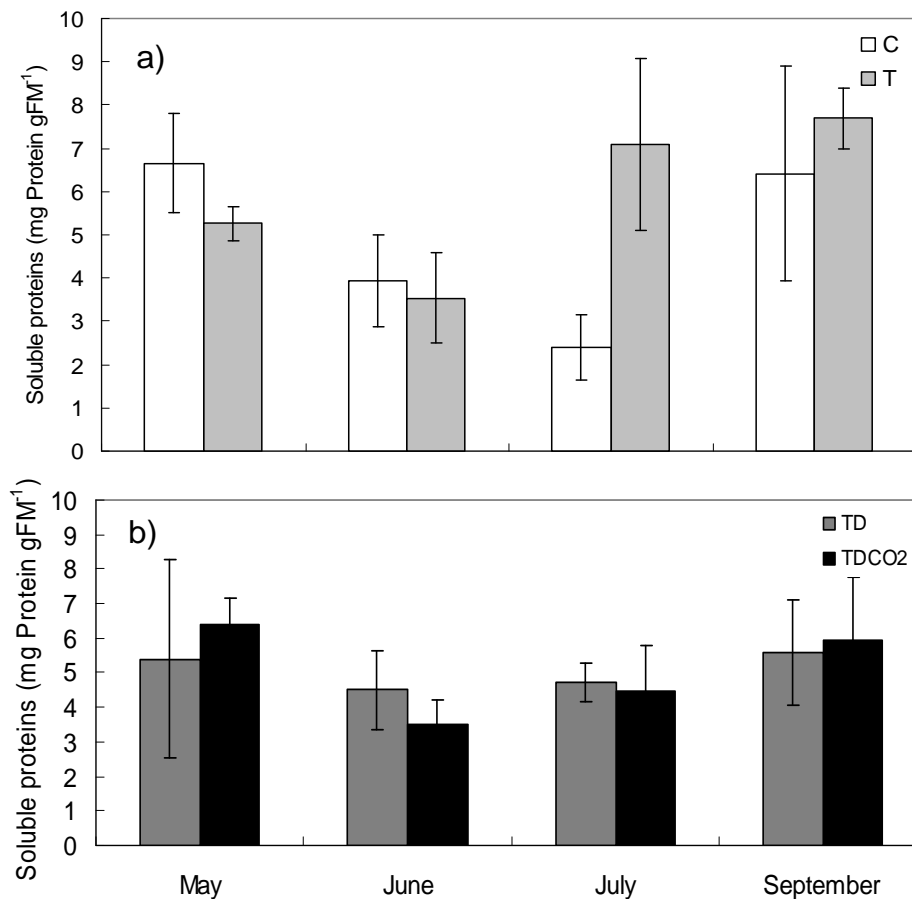
Overall, warming had a negative impact on leaf fructans concentrations (Table 3). Effects of warming on sucrose concentrations varied over time (significant Treatment x Date interaction;  $F_{1,4} = 2.7$ ,  $P < 0.1$ ). This Treatment x Date interaction seemed driven by a strong increase of sucrose concentrations in the T treatment compared to C treatment in September. Fructose concentrations also showed significant effects of warming, with an increase of fructose concentrations under T treatment for May, July and June (Table 3). Neither warming nor date had any significant effects on leaf glucose concentrations or stubble WSC concentrations (Table 3). Unlike warming, elevated  $CO_2$  had limited effects on leaf and stubble WSC concentration. Only fructose concentrations of leaves were positively affected by elevated  $CO_2$  treatment (Table 4).

#### ***4.6 Impacts of warming and elevated $CO_2$ on soluble leaf proteins, soluble and insoluble N and C***

Over the course of the experiment, soluble protein content ranged from 1.26 to 9.45 mg of protein by mg of fresh matter for leaf lamina across climate treatment (Figure 4). Total proteins showed a significant Treatment x Date interaction in response to warming ( $F_{1,4} = 3.9$ ,  $P < 0.05$ ). This was driven by a strong positive warming effect in July ( $F_{1,6} = 7.4$ ,  $P < 0.05$ , Figure 4a). Elevated  $CO_2$  had no significant effect on total proteins during the experiment (Figure 4b).

Across treatments and measurement dates, soluble N ranged from 0.06 to 0.61 %, insoluble N from 0.71 to 4.01 %, soluble C from 3.68 to 28.1 % and insoluble C from 38.6 to 48.3 % (Table 5 and Table 6). For leaf laminas, only insoluble C and N showed significant changes under warming (Table 5). Insoluble leaf N concentration showed a significant date effect ( $F_{1,4} = 3.6$ ,  $P < 0.001$ ) as a result of a decrease of insoluble N concentrations in June. Insoluble N concentration showed a tendency to decrease with warming ( $F_{1,2} = 3.2$ ,  $P < 0.1$ ; Table 5) but effects varied over time ( $F_{1,2} = 2.3$ ,  $P < 0.1$ ). This Treatment x Date interaction seemed driven by an increase of insoluble N in response to warming in September. In general, insoluble leaf C showed both a positive effect of warming and increases over the course of the

growing season (Table 5). Neither warming nor date had any significant effect on soluble C and N of leaves. For stubble, variations in insoluble N concentrations over time could be ranked: July > September > June and May (Table 5). Stubble insoluble N showed a tendency to increase under warming, but warming had no significant effect on soluble and insoluble stubble C concentrations (Table 5).



**Figure 4: Effects of warming (a) and elevated CO<sub>2</sub> (b) on soluble proteins of leaf from May to September 2009. Means and standard errors are presented per treatment and date (n = 12).**

Soluble C content of leaves in both TD and TDCO<sub>2</sub> showed a significant increase in June, leading to a significant effect of measurement date on leaf soluble C (Table 6). In contrast, insoluble leaf N significantly decreased in June (Table 6). Insoluble leaf N showed a tendency to decrease under elevated CO<sub>2</sub>, but elevated CO<sub>2</sub> had no significant effects on leaf insoluble C or N (Table 6). Both insoluble and soluble stubble N showed a significant negative response to elevated CO<sub>2</sub> but elevated CO<sub>2</sub> had no significant effect on either soluble or insoluble stubble C concentrations (Table 6).

## 5- Discussion

### 5.1 Warming effects on canopy- and leaf-level gas exchange and chemical composition

In line with the observation of a reduced above-ground production (see Cantarel et al., Chapter 1), canopy photosynthesis ( $A_{1000}$ ) showed a negative response to warming. Unsurprisingly, warming effects were strongest during the peak period of vegetative growth (May-June), and were of smaller magnitude during the reproductive / senescent vegetation phase typical of late summer for this grassland system. We found that canopy structure (i.e. LAI, faPAR and canopy height) was also negatively affected by warming, whereas radiation use efficiency (RUE) showed no differences between the warmed and control treatment. These results suggest that the observed decrease in photosynthetic rate in response to warming is closely linked to a reduction in the leaf area available for light interception. Light compensation point (LCP) showed no significant effects of warming suggesting that the canopy photosynthesis was activated at the same light level irrespective of warming (Ruimy *et al.* 1995). Photosynthetic responses to warming are known to depend on both the direct influence of elevated temperature and indirect effects of soil drying which usually occurs simultaneously (Shah & Paulsen 2003). In our study, control and warmed treatments received the same amount of water, reducing the potential for a water stress in the warmed treatment (T). Nevertheless, higher evapotranspiration and plant water-demand at the warmed site may have contributed to reductions in LAI during peak growing conditions (May). Consequently, warming effects may affect canopy photosynthesis indirectly by increasing water stress during the growing season, with cascading effects on canopy structure (leaf area and height) exacerbating the reduction in photosynthetic rates. Indeed, the ratio of precipitation to potential evapotranspiration was lower during the growing season in the T compared to the C treatment (Appendix 2).

Unlike, canopy-level measurements, warming did not have consistent effects on leaf-level photosynthetic responses of *F. arundinacea*. Positive effects of warming on Leaf $A_{1000}$  observed in June and September seemed to be linked with greater treatment differences in leaf temperature during photosynthesis measurements. Indeed, leaf-temperature differences between C and T were 3°C in June and September measurements but only  $\approx$  2°C at other

dates (data not shown). Smaller differences in leaf temperature between C and T treatments during photosynthesis measurements could result in smaller LeafA<sub>1000</sub> responses to warming treatment. Of course, 2 or 3 degrees difference in air temperature may have less impact on leaf lamina photosynthesis measured under favourable temperatures, compared with measurements carried out under more extreme conditions (cold or hot).

We found that Leaf A<sub>1000</sub> responses of *F. arundinacea* were not closely correlated with community-scale responses. Part of the discrepancy between these results could stem from methodological differences: leaf measurements were under favourable light and air temperatures whereas canopy measurements were made over a longer time period under variable temperature conditions. However, grass species are known to show significant interspecific variation in their photosynthetic capacity to warming (Niu et al. 2008), and canopy-level gas exchange were also driven by other dominant species in the canopy (e.g. *Elytrigia repens*, *Agrostis capillaris*). In the present study, we were only able to measure *F. arundinacea* leaves because of technical difficulties associated with the measurement of leaf-level photosynthesis for small-leaved graminoids of short stature. Further work is needed on other dominant species at our site to better understand the scaling-up of leaf-level processes to community-level responses.

In the above-ground vegetative tissues of temperate grasses, carbohydrate accumulation is directly related to the balance between current photosynthesis and overall sink demand (Humphreys *et al.* 2006). Our results suggested no changes in sink demand or accumulation of water-soluble carbohydrates (WSC) in *F. arundinacea* leaf stubble. Nevertheless, WSC in leaf laminae (i.e source) of *F. arundinacea* showed significant effects of warming, which varied depending on the identity of WSC. Fructans concentration was negatively affected by warming, whereas fructose and sucrose increased in response to warming. In addition, we found a tendency for increased insoluble C levels in *F. arundinacea* leaves under warming. This increase of insoluble C may be linked to an increase in leaf C storage *via* starch accumulation for plants growing under elevated temperature (Wilson & Bailey 1971). These changes were not correlated with LeafA<sub>1000</sub>, suggesting a long-term response of leaf carbohydrates compared to the leaf lamina photosynthesis responses (Chatterton *et al.* 1989). Changes in WSC under warming could reflect higher canopy evapotranspiration and leaf-level water stress under elevated temperature conditions.



Hydrolysis of fructan to fructose could increase osmotic potential of lamina cells and help minimise the negative effects of water stress in leaf cells (Wiemken *et al.* 1995; Hoekstra *et al.* 2001). Negative effects of warming on fructan concentrations could also be explained by the sensitivity of fructosyltransferases (involved with fructan synthesis) to elevated temperatures (Jeong & Housley 1990; Bancal & Triboi 1993).

### ***5.2 Elevated CO<sub>2</sub> effects on canopy- and leaf-level gas exchange and chemical composition***

Photosynthetic capacity of green shoots is known to increase under elevated CO<sub>2</sub> in the short-term (Arp & Drake 1991; Drake & Leadley 1991), although photosynthetic stimulation may decline over time due to accumulation of non-structural carbohydrates or resource constraints (Stitt & Krapp 1999; Luo *et al.* 2004). In our study we found that elevated CO<sub>2</sub> had a positive effect on canopy A<sub>1000</sub> early in the growing season, but no effect from July onwards. Furthermore, Km values indicated that canopy photosynthesis under elevated CO<sub>2</sub> was more efficient than the TD treatment in the beginning of the growing season. These results suggest that canopy photosynthesis in TDCO<sub>2</sub> treatments can occur with lower light conditions, and extend the period of positive carbon assimilation throughout the day and increase the rate of photosynthesis within the plant canopy (Drake & Leadley 1991; Long & Drake 1991). Canopy structure (i.e. LAI, faPAR) was also positively affected by elevated CO<sub>2</sub>, suggesting a better light interception under elevated CO<sub>2</sub>. It is possible that reduced CO<sub>2</sub> effects on canopy photosynthesis from July onwards indicate increasing acclimation to elevated CO<sub>2</sub>. Lower responsiveness from July onwards could stem: i) from increased downward regulation of leaf photosynthesis during summer and fall compared to spring; ii) from a phenological shift from photosynthetically-active, vegetatively-growing plants to a reproductive phase dominated by flowering individuals which tend towards senescence at the end of the summer.

Similar to canopy photosynthesis, leaf A<sub>1000</sub> measured for *F. arundinacea* declined significantly from spring (May) to summer. However, in contrast to canopy photosynthesis, the lack of stimulation of leaf photosynthesis occurred since the first measurement date and not only during late summer and fall. Discrepancies between CO<sub>2</sub>-induced stimulation of canopy- and of leaf-level photosynthesis is common because the photosynthetic capacity of the individual leaf is not the only factor which controls the photosynthetic rate of canopy (Ainsworth *et al.* 2003). Leaf area and geometry of the plant canopy, also determine the light interception characteristics of the canopy and thus the light intensity which the individual leaf

receives (Woledge & Leafe 1976). Of course, canopy-level responses reflect the photosynthetic responses of a complex, multi-species community and it is possible that the lack of stimulation of leaf photosynthesis by CO<sub>2</sub> began earlier in *F. arundinacea* compared to other dominant grass species. .

The effects of long-term exposure to CO<sub>2</sub>, compared to short-term responses, observed on *F. arundinacea* A<sub>1000</sub> during the period of vegetative growth are consistent with photosynthetic down-regulation or acclimation (Stitt & Krapp 1999). Previous work indicates that acclimation of leaf-level photosynthesis to elevated CO<sub>2</sub> is accompanied by higher carbohydrate concentration such as fructans (Casella & Soussana 1997) and a decrease in soluble proteins and RubisCO (Ainsworth & Long 2005). However, with the exception of fructose, we did not find any changes in leaf lamina WSC or soluble proteins in response to elevated CO<sub>2</sub>. Instead, elevated CO<sub>2</sub> was associated with increased insoluble N. Furthermore, we found a decrease in leaf and stubble insoluble N and a decrease in stubble soluble N concentrations under elevated CO<sub>2</sub>. Given that the plant N balance is known to control photosynthesis and plant growth acclimation under elevated CO<sub>2</sub> (Sage 1989; Drake *et al.* 1996), changes in N concentrations observed here may have been linked to *F. arundinacea* acclimation to elevated CO<sub>2</sub>. Further work should investigate the role of RubisCO for *F. arundinacea* acclimation to elevated CO<sub>2</sub>.

## 6- Conclusions

Our data indicate that warmed environments induce a decrease in canopy photosynthesis rates *via* changes in canopy structure with a decrease of LAI and height under elevated temperatures. Canopy-level patterns of photosynthesis in response to warming were different to *F. arundinacea* leaf photosynthetic rates which responded generally positively to warming. With the exception of fructans, leaf carbohydrate concentrations showed a positive response to warming in *F. arundinacea*, which may help buffer *F. arundinacea* leaves against increased water stress. Unlike warming, elevated CO<sub>2</sub> was associated with an initial increase in canopy-level photosynthetic rates and efficiency at low light levels during the period of vegetative growth (May-June). Canopy photosynthesis showed no response to elevated CO<sub>2</sub> in late summer, which may partly reflect a phenological shift the vegetation. *F. arundinacea* leaf photosynthetic rates showed no response to elevated CO<sub>2</sub> since spring and almost full leaf

photosynthetic acclimation to elevated CO<sub>2</sub> was observed. Patterns of CO<sub>2</sub> response in *F. arundinacea* were not directly linked to an accumulation of WSC in plants but appeared to be possibly caused by an increased ratio of soluble C to soluble N in leaves.. To better understand changes in leaf physiology, more specific experiments about the leaf photosynthetic response to CO<sub>2</sub> concentrations and light intensity would be needed. This would allow calculating important photosynthetic parameters such as maximum apparent carboxylation velocity ( $V_{c,max}$ ) and maximum rate of electron transport ( $J_{max}$ ) whose response to environmental variables has been shown to be very informative.

## **7- Acknowledgements**

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**Table 2: Effects of warming and elevated CO<sub>2</sub> treatment on leaf area index (LAI), radiation use efficiency (RUE), fraction of absorbed PAR (faPAR) and canopy height (means and standard errors are shown; n = 4). Results from repeated measures ANOVA testing the effects of warming or elevated CO<sub>2</sub>, measurement dates and their interaction are presented (significant P values are shown in bold).**

	Warming Effect (C vs T)					Elevated CO <sub>2</sub> Effect (TD vs TDCO <sub>2</sub> )						
	C	T	Model	Treatment	Date	Treatment x Date	TD	TDCO <sub>2</sub>	Model	Treatment	Date	Treatment x Date
<b>a) Leaf Area Index (LAI)</b>												
May	2.2 ± 0.2	1.2 ± 0.1	**	<0.001	0.707	0.517	1.0 ± 0.0	1.8 ± 0.4	ns	0.017	0.701	0.844
June	3.0 ± 0.5	1.4 ± 0.1					1.4 ± 0.1	2.6 ± 0.4				
July	3.2 ± 0.4	1.0 ± 0.1					1.1 ± 0.2	1.8 ± 0.3				
August	3.8 ± 0.6	0.9 ± 0.1					1.4 ± 0.4	1.7 ± 0.2				
September	2.9 ± 0.4	1.4 ± 0.1					1.3 ± 0.2	2.7 ± 0.7				
<b>b) Radiation Use Efficiency (RUE, mol CO<sub>2</sub> mol photon<sup>-1</sup>)</b>												
May	0.033 ± 0.000	0.027 ± 0.002	***	0.919	<0.001	<0.001	0.040 ± 0.002	0.043 ± 0.003	**	0.543	0.016	0.998
June	0.015 ± 0.001	0.014 ± 0.001					0.024 ± 0.005	0.020 ± 0.002				
July	0.024 ± 0.001	0.021 ± 0.001					0.036 ± 0.007	0.021 ± 0.001				
August	0.014 ± 0.000	0.021 ± 0.002					0.022 ± 0.004	0.016 ± 0.002				
September	0.011 ± 0.000	0.013 ± 0.000					0.012 ± 0.001	0.010 ± 0.000				
<b>c) Fraction of absorbed PAR (faPAR, %)</b>												
May	74.7 ± 7.1	49.1 ± 4.8	***	<0.001	0.217	0.382	45.3 ± 2.1	56.9 ± 13.3	ns	0.012	0.551	0.962
June	78.4 ± 6.1	56.3 ± 3.2					49.2 ± 8.9	72.8 ± 11.1				
July	79.3 ± 6.3	45.1 ± 5.9					42.3 ± 13.6	61.5 ± 10.9				
August	83.6 ± 7.3	43.9 ± 8.9					49.9 ± 17.3	61.1 ± 10.2				
September	84.1 ± 4.9	62.6 ± 6.7					56.3 ± 8.3	74.6 ± 12.2				
<b>d) Canopy height (cm)</b>												
May	31.5 ± 1.6	26.9 ± 0.9	***	0.016	<0.001	0.851	21.4 ± 1.5	22.4 ± 2.5	ns	0.171	<0.001	0.567
June	27.6 ± 1.7	30.7 ± 0.5					29.7 ± 2.7	29.8 ± 1.1				
July	33.1 ± 1.7	22.8 ± 0.6					22.1 ± 1.8	23.4 ± 0.9				
August	24.0 ± 1.9	20.5 ± 1.4					20.2 ± 1.6	21.1 ± 0.7				
September	21.9 ± 1.2	21.1 ± 0.7					21.2 ± 2.4	23.1 ± 0.9				

**Table 3: Effects of warming treatment on fructan, sucrose, glucose and fructose content for leaf lamina and stubble (means and standard errors are shown; n = 12). Results from repeated measures ANOVA testing the effects of warming, measurement dates and their interaction are presented (significant P values are shown in bold).**

	Leaf				Repeated measure ANOVA				Stubble				Repeated measure ANOVA			
	C		T		Model	Date	Treatment	Treatment x Date	C		T		Model	Treatment	Date	Treatment x Date
<b>a) Fructane (mg FA g DM<sup>-1</sup>)</b>																
May	89.8 ± 11.4	105.7 ± 34.5	**	<b>0.046</b>	0.055	0.285	233.5 ± 79.2	127.5 ± 17.7	ns	0.667	0.396	0.676				
June	198.2 ± 26.2	126.9 ± 30.0					258.0 ± 10.9	320.7 ± 25.9								
July	154.0 ± 29.7	126.5 ± 54.8					337.5 ± 18.3	252.4 ± 19.6								
September	158.5 ± 14.5	82.9 ± 18.8					265.4 ± 24.3	264.6 ± 29.3								
<b>b) Sucrose (mg SO g DM<sup>-1</sup>)</b>																
May	30.2 ± 0.6	32.5 ± 7.1	***	<b>0.021</b>	<b>0.002</b>	0.061	17.6 ± 4.1	16.2 ± 4.8	ns	0.420	0.102	0.852				
June	21.8 ± 4.3	24.2 ± 2.9					12.6 ± 2.1	18.3 ± 2.6								
July	14.7 ± 1.7	15.2 ± 4.4					10.4 ± 1.5	10.9 ± 1.5								
September	12.0 ± 3.0	30.7 ± 2.9					11.2 ± 1.9	15.2 ± 0.4								
<b>c) Glucose (mg GO g DM<sup>-1</sup>)</b>																
May	0.49 ± 0.05	1.1 ± 0.58	ns	0.115	0.108	0.245	2.7 ± 0.83	3.7 ± 1.4	ns	0.219	0.676	0.399				
June	0.57 ± 0.44	0.41 ± 0.14					1.0 ± 0.53	3.1 ± 1.6								
July	0 ± 0	0.09 ± 0.08					2.0 ± 0.41	1.7 ± 1.1								
September	0.2 ± 0.2	0.91 ± 0.18					1.3 ± 0.45	5.2 ± 3.4								
<b>d) Fructose (mg FO g DM<sup>-1</sup>)</b>																
May	4.0 ± 0.7	6.3 ± 1.1	*	<b>0.022</b>	0.201	<b>0.015</b>	7.6 ± 1.3	8.7 ± 3.2	ns	0.207	0.913	0.506				
June	6.1 ± 0.9	5.0 ± 0.4					5.0 ± 1.4	8.1 ± 2.8								
July	3.4 ± 0.5	5.0 ± 0.7					5.6 ± 1.0	7.2 ± 1.0								
September	4.6 ± 0.9	6.4 ± 0.5					3.8 ± 1.3	9.6 ± 4.1								

Table 4: Effects of elevated CO<sub>2</sub> treatment on fructan, sucrose, glucose and fructose for leaf lamina and stubble (means and standard errors are shown; n = 12). Results from repeated measures ANOVA testing the effects of elevated CO<sub>2</sub>, measurement dates and their interaction are presented (significant P values are shown in bold).

	Leaf				Stubble				
	TD		TDCO <sub>2</sub>		TD		TDCO <sub>2</sub>		
	Mean	SE	Model	Date	Mean	SE	Model	Date	
<b>a) Fructane (mg FA g DM<sup>-1</sup>)</b>									
May	64.9 ± 7.6	63.1 ± 5.7	**	0.384	151.2 ± 32.3	125.7 ± 39.2	*	0.284	0.897
June	121.1 ± 21.9	128.7 ± 16.0			271.8 ± 35.2	338.4 ± 14.9			
July	157.3 ± 9.9	176.9 ± 23.1			298.6 ± 11.9	323.4 ± 20.3			
September	88.9 ± 8.6	117.8 ± 39.8			248.6 ± 18.9	319.2 ± 24.7			
<b>b) Sucrose (mg SO g DM<sup>-1</sup>)</b>									
May	36.2 ± 14.2	26.9 ± 4.1	ns	0.141	12.5 ± 2.66	11.4 ± 3.5	*	0.356	0.706
June	24.5 ± 5.4	29.4 ± 5.7			13.4 ± 5.9	12.9 ± 3.4			
July	19.2 ± 1.9	29.3 ± 8.7			11.4 ± 2.1	10.9 ± 2.4			
September	18.3 ± 1.1	20.1 ± 4.7			12.3 ± 0.6	12.3 ± 1.7			
<b>c) Glucose (mg GO g DM<sup>-1</sup>)</b>									
May	1.1 ± 0.7	0.3 ± 0.1	**	0.402	2.3 ± 0.9	1.9 ± 1.0	ns	0.392	0.747
June	0.4 ± 0.1	1.1 ± 0.4			1.4 ± 0.9	0.9 ± 0.4			
July	0 ± 0	0 ± 0			1.4 ± 0.9	1.1 ± 0.3			
September	1.1 ± 0.1	1.2 ± 0.5			1.9 ± 1.1	1.6 ± 0.7			
<b>d) Fructose (mg FO g DM<sup>-1</sup>)</b>									
May	4.7 ± 1.7	5.1 ± 0.2	*	0.050	7.2 ± 0.8	6.6 ± 1.0	ns	0.209	0.581
June	4.3 ± 0.6	5.9 ± 0.5			5.4 ± 0.8	5.0 ± 1.3			
July	4.8 ± 0.5	5.0 ± 0.1			6.6 ± 1.1	5.4 ± 0.5			
September	6.1 ± 0.8	5.7 ± 0.7			8.5 ± 2.1	5.3 ± 1.9			

Table 5: Effects of warming treatment on soluble N, insoluble N, soluble C and insoluble C for lamina and stubble (means and standard errors are shown; n = 12). Significant P values ( $P < 0.05$ ) are shown in bold and marginal P values ( $0.1 > P > 0.05$ ) are in italics.

	Lamina				Stubble					
	C	T	Model	Repeated measure ANOVA Date Treatment	C	T	Model	Repeated measure ANOVA Date Treatment		
a) soluble N (%)										
May	0.14 ± 0.00	0.14 ± 0.05	ns	0.267	0.053	0.471	ns	0.100	0.399	0.493
June	0.12 ± 0.02	0.16 ± 0.04								
July	0.22 ± 0.01	0.21 ± 0.04								
September	0.15 ± 0.00	0.24 ± 0.03								
b) insoluble N (%)										
May	2.5 ± 0.2	2.3 ± 0.1	***	0.083	<b>&lt; 0.001</b>	0.095	**	0.084	<b>0.029</b>	0.131
June	1.8 ± 0.1	2.0 ± 0.2								
July	2.2 ± 0.3	2.3 ± 0.5								
September	2.3 ± 0.2	3.2 ± 0.5								
c) soluble C (%)										
May	12.4 ± 3.3	10.2 ± 2.2	ns	0.148	0.393	0.917	ns	0.596	0.984	0.514
June	14.1 ± 2.1	13.1 ± 1.6								
July	15.7 ± 4.1	13.1 ± 4.8								
September	13.1 ± 0.2	10.1 ± 0.9								
d) insoluble C (%)										
May	43.4 ± 0.4	44.1 ± 0.5	**	0.016	<b>0.001</b>	0.973	ns	0.541	0.262	0.688
June	44.9 ± 0.8	45.8 ± 0.5								
July	44.8 ± 0.1	45.6 ± 0.5								
September	45.1 ± 0.4	46.1 ± 0.6								

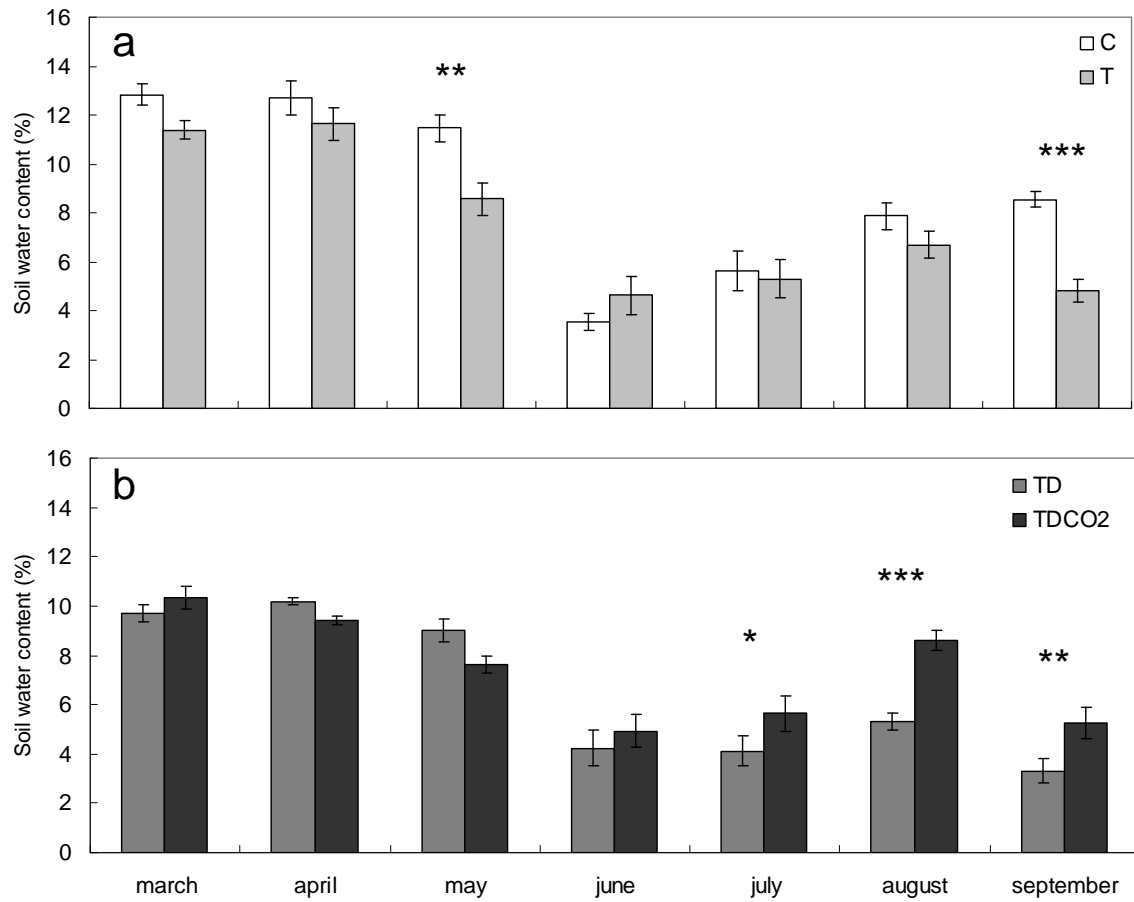
Table 6: Effects of elevated CO<sub>2</sub> treatment on soluble N, insoluble N, soluble C and insoluble C for lamina and stubble (means and standard errors are shown; n = 12). Significant P values (P < 0.05) are shown in bold and marginal P values (0.1 > P > 0.05) are in italics.

	Lamina				Stubble							
	TD	TDCO <sub>2</sub>	Model	Repeated measure ANOVA Date Treatment x Date	TD	TDCO <sub>2</sub>	Model	Repeated measure ANOVA Date Treatment x Date				
a) soluble N (%)												
May	0.17 ± 0.02	0.17 ± 0.03	ns	0.747	0.488	0.372	0.13 ± 0.02	0.19 ± 0.08	*	0.015	0.018	0.217
June	0.15 ± 0.03	0.25 ± 0.11					0.25 ± 0.06	0.12 ± 0.01				
July	0.15 ± 0.00	0.20 ± 0.03					0.24 ± 0.06	0.11 ± 0.04				
September	0.31 ± 0.04	0.21 ± 0.02					0.47 ± 0.13	0.26 ± 0.07				
b) insoluble N (%)												
May	3.3 ± 0.2	2.7 ± 0.3	***	0.072	< 0.001	0.278	1.1 ± 0.1	1.1 ± 0.2	ns	0.023	0.407	0.764
June	1.8 ± 0.2	1.8 ± 0.2					1.1 ± 0.0	0.8 ± 0.1				
July	2.3 ± 0.1	2.3 ± 0.0					1.2 ± 0.1	0.9 ± 0.1				
September	3.3 ± 0.5	2.8 ± 0.4					1.3 ± 0.0	1.1 ± 0.1				
c) soluble C (%)												
May	9.7 ± 0.9	8.7 ± 1.1	*	0.492	0.019	0.401	8.3 ± 2.3	7.0 ± 1.5	ns	0.286	0.419	0.741
June	11.3 ± 2.2	14.7 ± 3.2					19.3 ± 0.8	16.0 ± 4.2				
July	6.6 ± 1.2	9.7 ± 1.5					11.3 ± 4.2	10.5 ± 5.0				
September	8.8 ± 0.7	8.2 ± 1.3					17.1 ± 2.5	16.1 ± 4.2				
d) insoluble C (%)												
May	43.7 ± 0.2	44.3 ± 0.6	ns	0.952	0.263	0.952	46.3 ± 0.7	45.3 ± 1.5	ns	0.505	0.281	0.826
June	44.3 ± 1.5	44.3 ± 1.6					45.2 ± 1.1	44.0 ± 0.0				
July	45.3 ± 0.4	44.9 ± 0.2					45.3 ± 1.2	45.0 ± 0.7				
September	46.0 ± 0.4	45.7 ± 0.7					45.0 ± 0.8	44.2 ± 0.6				

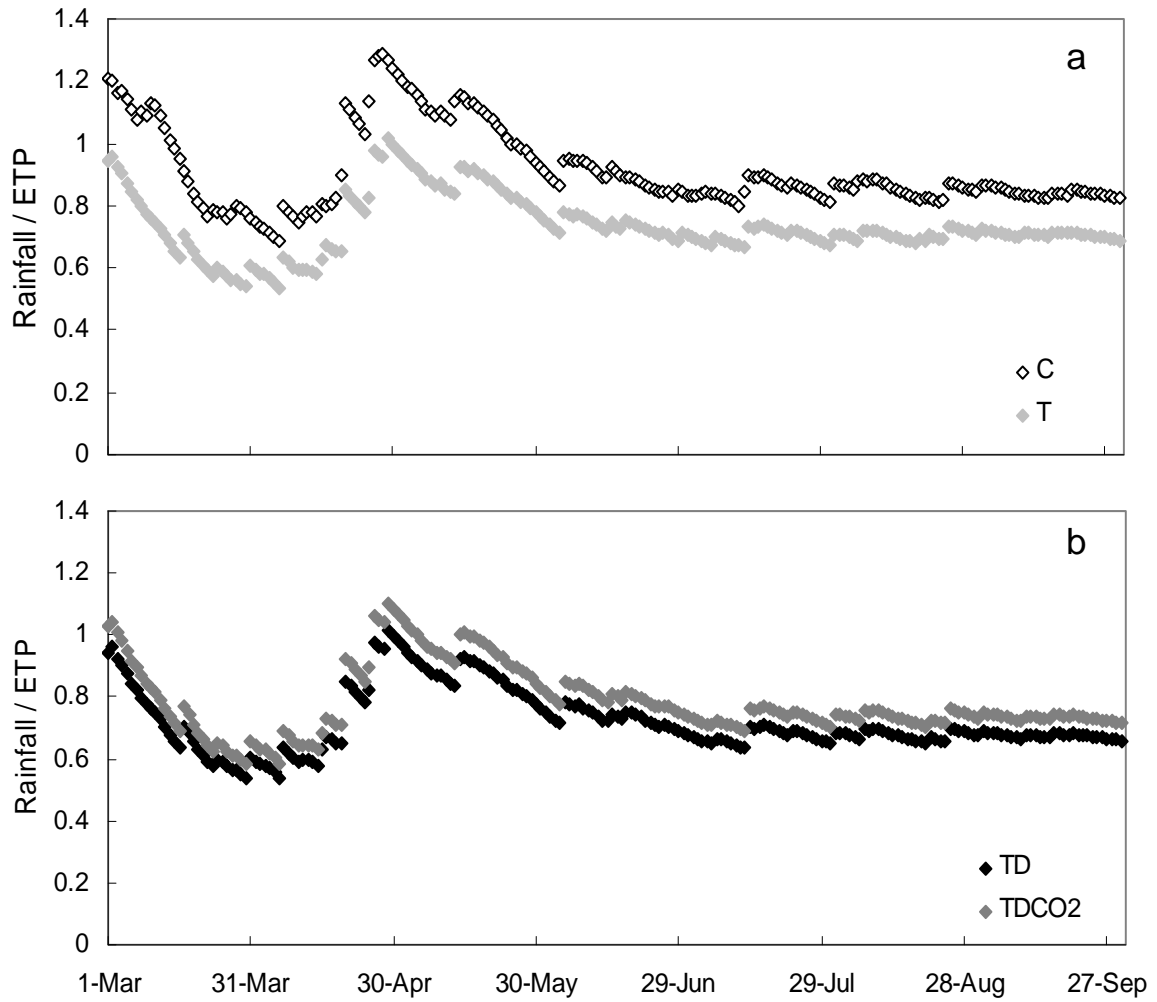


## 8- Appendix

Appendix 1: Monthly mean of soil water content (%) from March to September 2009 for (a) C and T treatments and for (b) TD and TDCO<sub>2</sub> treatments



Appendix 2: Cumulative rainfall divided by cumulative potential evapotranspiration (Rainfall / ETP) from March to September 2009 for C and T treatments (a) and TD and TDCO<sub>2</sub> treatments (b)





## ***Résultats - Chapitre 3***

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### **Effets des facteurs (i.e. température, CO<sub>2</sub> et sécheresse) du changement climatique sur les flux d'oxyde nitreux (N<sub>2</sub>O) en prairie.**

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Ce chapitre cherche à caractériser les flux de N<sub>2</sub>O en prairie et ses dynamiques saisonnières et annuelles sous changement climatiques. Il s'attache aussi à étudier les relations entre les flux de N<sub>2</sub>O et les facteurs abiotiques tels que la température du sol et la teneur en eau du sol. Il est présenté sous forme d'un article qui a été accepté en 2010 dans la revue '*Ecosystems*'. Il s'intitule : 'Effects of climate change drivers on nitrous oxide fluxes in an upland temperate grassland.' Les co-auteurs sont Juliette Bloor<sup>a</sup>, Nicolas Deltruy<sup>b</sup> et Jean-François Soussana<sup>c</sup>.

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a : INRA - UR 874 Clermont-Ferrand, France

b : Département Génie Industriel et Maintenance, Ensemble Universitaire des Cézeaux Aubière, France

c : INRA - CODIR - ENVIRONNEMENT Paris, France

## Plan du Chapitre 3 des résultats

1- Abstract

2- Introduction

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*3.1 Study site and experimental design*

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## **1- Abstract**

Despite increasing interest in the patterns of trace gas emissions in terrestrial ecosystems, little is known about the impacts of climate change on nitrous oxide (N<sub>2</sub>O) fluxes. The aim of this study was to determine the importance of the three main drivers of climate change (warming, summer drought and elevated CO<sub>2</sub> concentrations) on N<sub>2</sub>O fluxes from an extensively-managed, upland grassland. Over a two-year period, we monitored N<sub>2</sub>O fluxes in an in situ ecosystem manipulation experiment simulating the climate predicted for the study area in 2080 (3.5 °C temperature increase, 20 % reduction in summer rainfall and atmospheric CO<sub>2</sub> levels of 600 ppm). N<sub>2</sub>O fluxes showed significant seasonal and interannual variation irrespective of climate treatment, and were higher in summer and autumn compared with winter and spring. Overall, N<sub>2</sub>O emissions showed a positive correlation with soil temperature and rainfall. Elevated temperature had a positive impact on mean annual N<sub>2</sub>O fluxes but effects were only significant in 2007. Contrary to expectations, neither combined summer drought and warming nor the simultaneous application of elevated atmospheric CO<sub>2</sub> concentrations, summer drought and warming had any significant effect on annual N<sub>2</sub>O fluxes. However, the maximum N<sub>2</sub>O flux rates observed during the study occurred when elevated CO<sub>2</sub> was combined with warming and drought, suggesting the potential for important, short-term N<sub>2</sub>O-N losses in enriched CO<sub>2</sub> environments. Taken together, our results suggest that the N<sub>2</sub>O responses of temperate, extensively-managed grasslands to future climate change scenarios may be primarily driven by temperature effects.



## 2- Introduction

Nitrous oxide (N<sub>2</sub>O) is both an influential greenhouse gas, with a global warming potential ~300 times that of CO<sub>2</sub> (IPCC 2001), and the single most important ozone-depleting component in present times (Ravishankara *et al* 2009). The atmospheric concentration of this trace gas has shown linear increases of 0.2-0.3 % y<sup>-1</sup> over the last few decades, largely as a result of changes in agricultural practices and direct emissions from agricultural soils (IPCC 2007). For example, the addition of synthetic or organic N fertilizers in managed lands generally increases N<sub>2</sub>O emissions (Granli & Bøckman 1994). However, N<sub>2</sub>O emissions also show strong variability in response to changes in environmental conditions i.e. temperature and rainfall (Smith *et al* 1998; Dobbie & Smith 2001; Flechard *et al* 2007). Given the growing consensus that global climate is changing rapidly (IPCC 2007), climate-driven changes in N<sub>2</sub>O emissions could play an increasingly important role in the pattern of future atmospheric N<sub>2</sub>O concentrations and the possible feedbacks to global warming.

In terrestrial ecosystems, the majority of N<sub>2</sub>O production results from microbial nitrification and denitrification in soils (Bremner 1997; Wrage *et al* 2004). The relative contribution of these two processes, and the magnitude of N<sub>2</sub>O emissions, depends largely on soil physical parameters including soil temperature, oxygenation, mineral nitrogen and water content (Smith *et al* 2003; Jones *et al* 2005). Recent work on European permanent grasslands has shown that 48 % of the temporal variability in N<sub>2</sub>O fluxes across sites can be explained by soil temperature and moisture, with increased N<sub>2</sub>O production in warm, wet environments (Fléchard *et al* 2007). Clearly, rising temperature and shifts in rainfall associated with rising greenhouse gas concentrations, particularly atmospheric carbon dioxide (CO<sub>2</sub>), may be expected to alter N<sub>2</sub>O-producing soil processes, both directly and indirectly via changes in plant biomass, root exudates or community structure which modify soil moisture conditions (Hungate *et al* 2003; Niklaus *et al* 2006; Zak *et al* 2000). In addition, elevated CO<sub>2</sub> may itself affect soil N transformations and gaseous N<sub>2</sub>O losses as a result of increased carbon substrate availability and changes in plant-soil water relations (Luo & Mooney 1999). Previous studies in grassland communities have found conflicting responses of N<sub>2</sub>O emissions to elevated CO<sub>2</sub>, reporting an increase (Baggs *et al* 2003), decrease (Kettunen *et al* 2005) or no effect of CO<sub>2</sub> treatment (Billings *et al* 2002) on N<sub>2</sub>O fluxes. This discrepancy between studies may in part reflect differences in experimental design (Kammann *et al* 2008). However, despite the



importance of multiple climate change drivers for N<sub>2</sub>O production, no studies to date have investigated the impacts of projected climate change scenarios on N<sub>2</sub>O emissions.

In the present paper, we examine the effects of warming, elevated CO<sub>2</sub> and summer drought on N<sub>2</sub>O fluxes on an upland grassland, making use of the Clermont Climate Change Experiment facility, a long-term grassland study of multiple climate changes applied in an additive experimental design (Bloor *et al* 2010). Our extensively-managed grassland study system is of particular interest because i) extensification of grassland management is an increasingly common land-use change in the European Union, promoted by agri-environmental schemes (Marriott *et al* 2009), and ii) unlike intensively-managed agricultural grasslands, literature on N<sub>2</sub>O production in unproductive grasslands is relatively scarce (Kammann *et al* 1998; Flechard *et al* 2007). N<sub>2</sub>O fluxes were measured over a two-year period to investigate seasonal and interannual variation in N<sub>2</sub>O production and to determine how N<sub>2</sub>O fluxes respond to either elevated temperature, combined elevated temperature and summer drought, or combined elevated temperature, summer drought and elevated CO<sub>2</sub>. We hypothesized that N<sub>2</sub>O emissions would increase in response to elevated temperature, with strongest effects being observed in spring and autumn under favorable soil moisture conditions. We also predicted that 1) N<sub>2</sub>O emissions would decrease in response to combined summer drought and warming, compared with warming alone, and 2) improved plant water relations and soil moisture under elevated CO<sub>2</sub> would result in greater N<sub>2</sub>O emissions under simultaneous warming, summer drought and elevated CO<sub>2</sub> compared with combined summer drought and warming under ambient CO<sub>2</sub> levels. Finally, we tested whether climate change treatments modified the relationships between abiotic factors (soil temperature, soil moisture) and N<sub>2</sub>O fluxes.

### **3- Materials and methods**

#### ***3.1 Study site and experimental design***

The study site is located in an upland permanent grassland in the French Massif Central region (Theix, 45°43'N, 03°01'E, 850 m a.s.l.). The site is characterised by a Cambisol soil (59.5 % sand, 19.7 % silt, 20.8 % clay, pH<sub>H2O</sub> 6.2), and a plant community dominated by grasses (*Festuca arundinaceae*, *Elytrigia repens*, *Poa pratensis*). Mean annual temperature at the site is 8.7°C and mean annual precipitation is 780mm.

The field experiment (Clermont Climate Change Experiment, Bloor *et al* 2010) was initiated in May 2005, manipulating air temperature, summer rainfall and atmospheric CO<sub>2</sub> in an additive, replicated design. The design consisted of 80 grassland monoliths (0.5 x 0.5 x 0.4 m in size), excavated from the study site and allocated at random to one of four climate treatments; C (control), T (+ 3.5 °C), TD (+ 3.5 °C, 20 % reduction in summer rainfall) and TDCO<sub>2</sub> (+ 3.5 °C, 20 % reduction in summer rainfall, CO<sub>2</sub> levels of 600 ppm). Four monoliths were grouped together to form a single experimental unit (five experimental units per climate treatment); units were placed in pre-prepared holes in the ground such that the surface of all monoliths was on a level with the surrounding soil surface (for full details see Bloor *et al* 2010).

Climate change treatments (T, TD and TDCO<sub>2</sub>) were based on IPCC predictions for the French Massif Central region for 2080 (ACACIA A2 scenario, IPCC 2001). Elevated temperatures were achieved by transporting monoliths to a lower-altitude site 14 km away (Clermont-Ferrand, 45°47'N, 03°05'E, 350m a.s.l.). The lowland site is characterised by a mean annual temperature of 11.1 °C and a mean annual rainfall of 374 mm (38 year averages). Given consistently lower monthly rainfall levels at the lowland site (-18.3 ± 4.1 mm), rainfall in the T treatment was supplemented by irrigation to generate similar rainfall patterns to the C treatment in upland site. Summer drought in the TD treatment was obtained by a reduction of supplementary water levels during June, July and August. Mini-FACE (Free Air Carbon dioxide Enrichment) technology was used to establish the elevated CO<sub>2</sub> treatment; the target CO<sub>2</sub> concentration was operational during daylight hours and was maintained via input/output signals at an automated control system.

From April 2005 onwards, all grass monoliths in the different climate treatments were cut to a height of 5 cm at six-month intervals (April and October). Monoliths were left to grow without the addition of fertiliser. Climate measurements were made using a Campbell Scientific automatic weather station and logged to a CRX-10 data logger (Campbell scientific Inc., Utah, USA) at 30 minute intervals for both the upland and lowland sites. Volumetric soil moisture (0-20 cm) was recorded hourly in each climate treatment using ECH<sub>2</sub>O-20 probes (Dielectric Aquameter, Decagon Devices, Inc., Pullman, WA; n = 3). Soil temperature in the topsoil layer (2-5 cm) of each climate treatment was recorded by thermocouples (TC S.A., Dardilly, France; n = 5).

### 3.2 N<sub>2</sub>O flux measurements

Nitrous oxide fluxes were measured on average twice a month from 06 March 2007 to 27 February 2009 using non-vented closed manual chambers on one monolith per experimental unit in all climate treatments ( $n = 5$ ). Four months prior to the start of N<sub>2</sub>O measurements, a permanent stainless steel base was installed around each target monolith (14 cm high, inserted 10 cm into the soil); the upper rim had a rubber gasket to ensure a tight seal. Two sizes of opaque PVC chambers were used for N<sub>2</sub>O measurements during the study to allow for seasonal variation in vegetation height (small: 0.5 x 0.5 x 0.15 m; large: 0.5 x 0.5 x 0.4 m); the outer surface of the chambers were coated in a reflective film to minimise chamber warming during flux measurements. At each measurement date, N<sub>2</sub>O measurements were carried out over the morning and afternoon, avoiding the hot midday period (12 a.m. – 3 p.m.) during the summer months. Emissions of N<sub>2</sub>O from soil are known to show temporal and diurnal variations (Smith *et al* 1998). To minimise possible confounding effects of diurnal patterns on N<sub>2</sub>O emissions in the different climate treatments, the timing (morning/ afternoon) of flux measurements was alternated for different repetitions within each climate treatment.

During N<sub>2</sub>O measurement campaigns, chambers were fixed onto the permanent bases for each target monolith and 180 ml gas samples were taken at five twenty-minute intervals using a quick release pneumatic connector (TST Tansam Inc, Kocaeli, Turkey) and a PTFE-Teflon tube connected to an INNOVA 1412 photoacoustic multi-gas analyser (INNOVA AIR Tech Instruments, Ballerup, Denmark). This sample removal rate was considered to have negligible effects on the air pressure within the chamber. After each sample, ambient air was flushed through the INNOVA to avoid contamination between measurements. N<sub>2</sub>O fluxes rates ( $\mu\text{g N}_2\text{O-N m}^{-2} \text{ h}^{-1}$ ) were calculated from the linear increase or decrease of the N<sub>2</sub>O concentration in the chamber against time (Schrier-Ujil *et al*, 2010). N<sub>2</sub>O flux events were recorded, if significant N<sub>2</sub>O regressions were obtained ( $P < 0.05$ ,  $r^2 \geq 0.95$ ). Regressions of CO<sub>2</sub> concentration versus time were also used as a quality control criterion; flux data points were rejected if the  $r^2$  for CO<sub>2</sub> fell below 0.95. In total, 40 measurement campaigns were carried out over the 2-year period (21 and 19 in 2007 and 2008 respectively).

The INNOVA gas analyser was encased in an air-conditioned box to avoid confounding effects of temperature on analyser measurements. Cross-interference between CO<sub>2</sub> and N<sub>2</sub>O was minimised using automatic cross-compensation procedures within the INNOVA (based on an optical filter and gas calibration with pure gas standards). As an

additional safeguard, N<sub>2</sub>O measurements with high CO<sub>2</sub> concentration at the end of the measurement campaign were rejected (CO<sub>2</sub> > 1800 ppm, 15 % of total campaigns). On average, the maximal CO<sub>2</sub> concentrations reached 1312 ppm ± 12 ppm across treatments and measurement campaigns (n = 800), and showed no relationship with either absolute N<sub>2</sub>O values or N<sub>2</sub>O flux values over the experimental period. Maximal CO<sub>2</sub> emissions recorded in the chambers showed no significant effects of Treatment or Year and there were no significant Treatment x Year interactions (repeated measures ANOVA, P>0.1). Regular checking and calibration of N<sub>2</sub>O and CO<sub>2</sub> measurements throughout the study period ensured minimal drift for N<sub>2</sub>O and CO<sub>2</sub> measurements (< 5%).

### 3.3 Statistical analysis

Effects of climate treatment on N<sub>2</sub>O fluxes were analysed using repeated measures analysis of variance (ANOVA) with both treatment and year as a fixed factor (Zar, 1998). Interactive effects of season and climate treatment were assessed separately for 2007-8 and 2008-9 using repeated measures ANOVA with treatment and season as a fixed factor. Climate effects for individual measurement campaigns were analysed using log-transformed N<sub>2</sub>O data and one-way ANOVA or non-parametric Kruskal-Wallis tests when data did not conform with assumptions of normality and homogeneity of variances. For all analyses, *a priori* comparisons examining the effects of climate change drivers (temperature, drought, CO<sub>2</sub>) were carried out using orthogonal contrasts.

Relationships between N<sub>2</sub>O fluxes and abiotic factors were examined using Pearson correlation coefficients, calculated between mean values of soil temperature, soil moisture, rainfall and N<sub>2</sub>O flux values per climate treatment for each measurement campaign. Multiple regression analysis was used to identify linear relationship between fluxes and abiotic factors for each climate treatment; N<sub>2</sub>O flux data were linearised using a monotonic transformation such that the distribution minimum was anchored at 1 (Osborne, 2002) and all other data were log-transformed prior to analysis. Stepwise regressions were used to identify variables which maximize the coefficient of determination (r<sup>2</sup>) by an automatic procedure. All analysis was carried out using Statgraphics Plus 4.1 (Statistical Graphics Corp., Rockville, Maryland, USA).

## 4- Results

### *4.1 Meteorological conditions and climate treatments*

Over the course of the study period, the difference between the mean monthly temperature in the control and elevated temperature treatments varied between 2 and 3.8 °C (average difference  $3.3 \pm 0.67$  °C, Appendix 1). Differences in precipitation between C and T treatments were effectively minimized by supplemental watering (mean annual difference  $20.8 \pm 3.9$  mm, < 2.5 % of total rainfall). In summer (June, July, August), the drought (TD, TDCO<sub>2</sub>) treatments received a 22 % reduction in rainfall compared with the T treatment for both 2007 and 2008. Mean daily values of CO<sub>2</sub> in the TDCO<sub>2</sub> treatment over the study period were  $583.4 \pm 3.2$  ppm compared with  $380 \pm 3.1$  ppm recorded in ambient CO<sub>2</sub> conditions. No significant differences in ambient CO<sub>2</sub> values were found between upland site (C treatment) and lowland site (T and TD treatment) during the study period.

Microclimatic variables recorded on days of N<sub>2</sub>O measurements indicated no difference in soil moisture conditions (WFPS) between the C and T treatments during the study period ( $F_{1,79} = 1.23$ ,  $P > 0.05$ , Table 1). In contrast, the warmed, drought treatments had lower WFPS compared with the warmed treatment alone ( $F_{1,79} = 8.6$  and  $F_{1,79} = 9.1$ ,  $P < 0.05$ , for TD and TDCO<sub>2</sub> versus T respectively). No WFPS differences were observed between the TD and TDCO<sub>2</sub> treatments across measurement campaigns ( $F_{1,79} = 0.12$ ,  $P > 0.05$ ; Table 1). In general, variation in WFPS and temperature recorded during N<sub>2</sub>O measurement campaigns mirrored patterns observed at the seasonal scale based on continuous microclimate data. The one exception was found in summer 2007, where average WFPS recorded during N<sub>2</sub>O measurement campaigns was lower than the seasonal average based on continuous measurements (-20%). This resulted from logistical difficulties measuring N<sub>2</sub>O fluxes during the particularly rainy summer of 2007.

**Table 1.** Air temperature, soil temperature and WFPS recorded during the N<sub>2</sub>O campaign measurements for each treatment in 2007 and 2008. Means and standard errors are presented per treatment (n = 105 and 95 for 2007 and 2008 respectively).

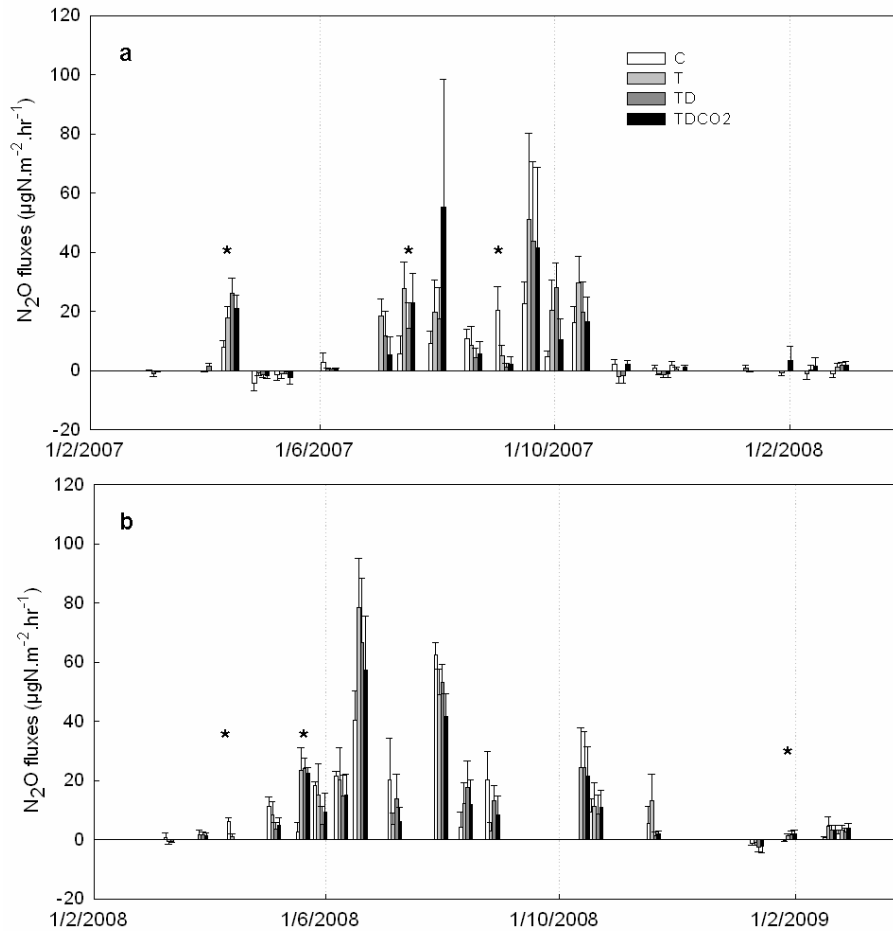
	Treatment	Air Temp. (°C)	Soil Temp. (°C)	WFPS (%)
2007	C	11.5 ± 1.23	10.1 ± 1.42	61.2 ± 1.63
	T	15.3 ± 1.60	12.9 ± 1.46	60.2 ± 3.06
	TD	15.3 ± 1.60	13.2 ± 1.44	48.2 ± 3.07
	TDCO <sub>2</sub>	15.3 ± 1.60	13.1 ± 1.40	50.6 ± 2.53
2008	C	14.6 ± 1.62	11 ± 1.46	63.8 ± 2.53
	T	16.1 ± 1.67	12.6 ± 1.67	60.7 ± 3.75
	TD	16.1 ± 1.67	12.7 ± 1.65	53.9 ± 3.11
	TDCO <sub>2</sub>	16.1 ± 1.67	12.8 ± 1.64	53.3 ± 3.63

*Treatment codes are given by: C = control; T = elevated; TD = elevated temperature and summer drought; TDCO<sub>2</sub> elevated temperature, summer drought and CO<sub>2</sub> enrichment*

#### 4.2 Effects of climate change drivers on N<sub>2</sub>O fluxes

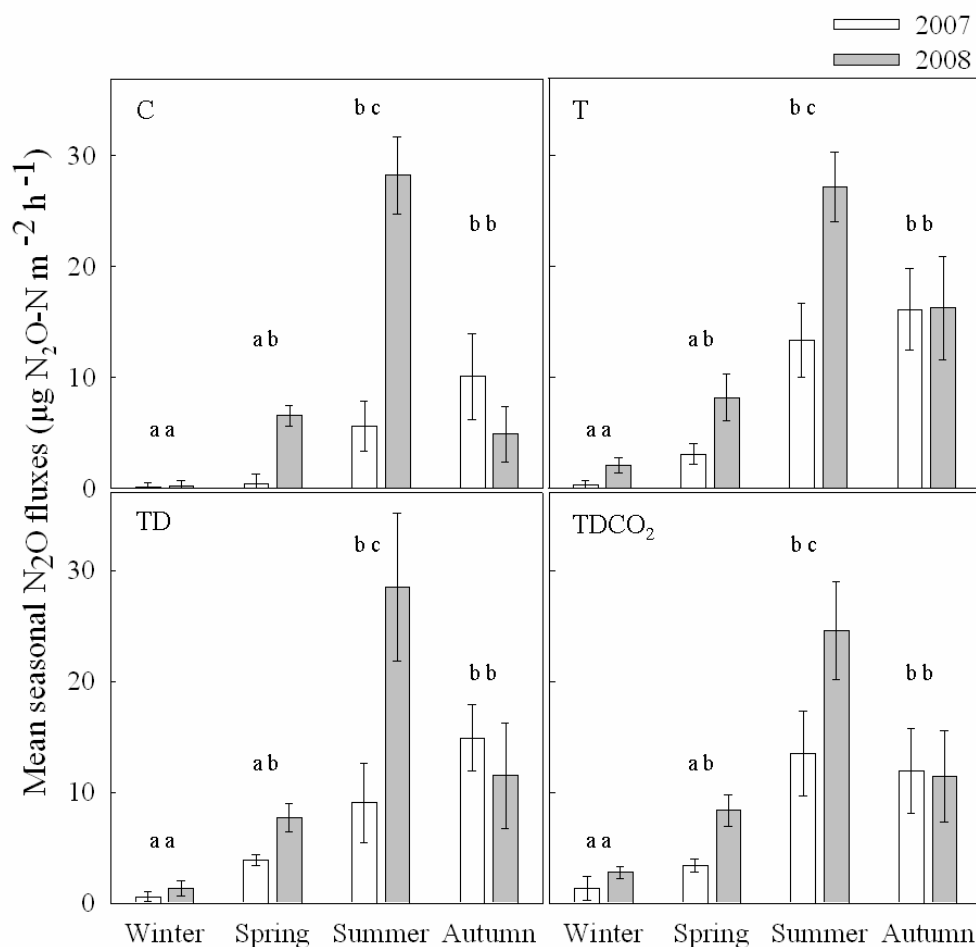
Over the course of the study, N<sub>2</sub>O fluxes ranged from -13.1 to 203.3 μg N<sub>2</sub>O-N m<sup>-2</sup> hr<sup>-1</sup> across treatments and showed significant variation between measurement campaigns (Figure 1). N<sub>2</sub>O uptake events corresponded to 6% of the total number of measurements across treatments, and were found during winter and spring only.

Effects of climate treatment on the magnitude of N<sub>2</sub>O emissions were difficult to detect due to high variation in N<sub>2</sub>O fluxes within treatments (Figure 1). During the study, only six out of forty measurement campaigns showed significant temperature effects (Figure 1). These temperature effects were not consistent, since both increases and decreases in N<sub>2</sub>O emissions were found in the T compared to the C treatment in both 2007 and 2008 (Figure 1). Neither summer drought under warmed conditions (T versus TD) nor elevated CO<sub>2</sub> in combination with warming and summer drought (TD versus TDCO<sub>2</sub>) had any significant effect on N<sub>2</sub>O emissions recorded for individual measurement campaigns.



**Figure 1.** Effects of climate treatment on N<sub>2</sub>O fluxes for measurement campaigns (a) from March 2007 to March 2008 and (b) from March 2008 to March 2009. Means and standard errors are shown ( $n = 5$ ). Asterisks indicate campaigns with significant treatment effects (data natural log transformed; Kruskal Wallis test,  $P < 0.05$ ).

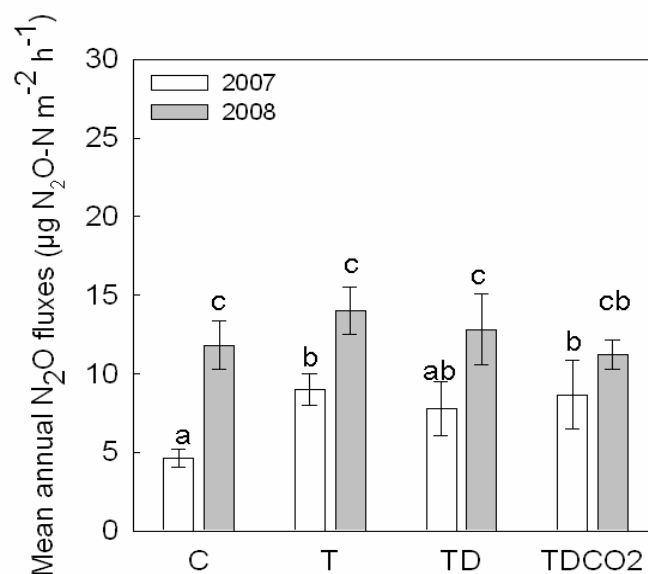
In general, greater N<sub>2</sub>O emissions were found in the summer months whereas smaller N<sub>2</sub>O emissions or/and uptakes were found over the winter and spring (Figure 2). Climate treatments had limited effects on mean seasonal N<sub>2</sub>O fluxes; in 2007, elevated temperature had a positive effect on mean N<sub>2</sub>O emissions in spring (C versus T,  $F_{1,16} = 18.3$ ,  $P < 0.01$ ), but no other treatment effects were detected during seasons. Irrespective of climate treatment, N<sub>2</sub>O emissions were greater in the spring and summer of 2008 compared with 2007 (Figure 2).



**Figure 2.** Seasonal variation in N<sub>2</sub>O fluxes for experimental climate treatments during the two year study period. Treatment codes are given by: C = control; T = elevated temperature; TD = elevated temperature and summer drought; TDCO<sub>2</sub> = elevated temperature, summer drought and CO<sub>2</sub> enrichment. Means and standard errors are presented (n = 5); different letters indicate a significant difference (P < 0.05) based on LSD-tests.

Over the study period, mean annual N<sub>2</sub>O fluxes showed a significant effect of both treatment and year (repeated measures ANOVA,  $F_{3,16} = 2.9$ ,  $P < 0.05$  and  $F_{1,4} = 28.4$ ,  $P < 0.001$  respectively). Effects of treatment did not differ significantly between years (no significant Treatment x Year interaction), and experimental warming was found to have a positive effect on mean annual N<sub>2</sub>O emissions overall. Nevertheless, the positive effect of elevated temperature on mean annual N<sub>2</sub>O fluxes was stronger in 2007 compared with 2008 (C versus T,  $F_{1,16} = 3.75$ ,  $P < 0.05$  in 2007; non-significant positive tendency in 2008; Figure 3). Mean annual N<sub>2</sub>O emissions showed no response to summer drought under warmed conditions (T versus TD) or to elevated CO<sub>2</sub> under warmed conditions coupled with summer drought (TD versus TDCO<sub>2</sub>) in either 2007 or 2008 (Figure 3)





**Figure 3.** Effects of climate treatment on mean annual N<sub>2</sub>O fluxes (different letters indicate significant differences ( $P < 0.05$ ) based on LSD-tests). Treatment codes are given by: C = control; T = elevated temperature; TD = elevated temperature and summer drought; TDCO<sub>2</sub> = elevated temperature, summer drought and CO<sub>2</sub> enrichment. Means and standard errors are presented ( $n = 5$ ).

#### 4.3 Relationship of N<sub>2</sub>O fluxes with abiotic factors (soil temperature, rainfall and WFPS)

The magnitude N<sub>2</sub>O emissions or N<sub>2</sub>O uptakes showed no significant treatment differences along a gradient of soil temperature or soil moisture (Figure 4). Furthermore, relationships between N<sub>2</sub>O fluxes and abiotic factors were not significant within each climate treatment. Greatest N<sub>2</sub>O emissions were recorded at soil temperatures between 15 °C and 25 °C for all climate treatments (Figure 4).

Across treatments, N<sub>2</sub>O emissions showed a positive correlation with soil temperature ( $r = 0.643$ ,  $P < 0.01$ ,  $n = 160$ ) and daily rainfall ( $r = 0.417$ ,  $P < 0.01$ ,  $n = 160$ ) but a negative correlation with soil WFPS ( $r = -0.250$ ,  $P < 0.05$ ,  $n = 160$ ) during the study period. However, stepwise multiple regressions showed that the relative importance of these abiotic drivers of N<sub>2</sub>O fluxes varied depending on climate treatment (Table 2). In the control treatment, N<sub>2</sub>O fluxes showed a significant relationship with soil temperature alone, but in the T treatment, N<sub>2</sub>O fluxes showed a relationship with precipitation and soil WFPS (Table 2). The TD treatment fluxes showed a relationship with all abiotic factors whereas TDCO<sub>2</sub> treatment fluxes seemed to be best explained by soil temperature and soil WFPS.

Table 2. Multiple linear regression analysis of N<sub>2</sub>O fluxes against soil temperature, soil moisture and rainfall.

Climate treatment	r <sup>2</sup>	P-model	Soil temperature (°C)	WFPS (%)	Rainfall (mm)
C	18.97	0.010	0.35**	NS	NS
T	47.93	0.001	NS	-1.30**	0.64***
TD	37.77	0.006	0.25*	-0.84*	0.27*
TDCO <sub>2</sub>	32.32	0.008	0.48***	-0.71*	NS

Correlation coefficients are shown for each variable. NS not significant. \*P<0.05, \*\*P<0.01, \*\*\*P<0.001  
 Treatment codes are given by: C = control; T = elevated; TD = elevated temperature and summer drought; TDCO<sub>2</sub> elevated temperature, summer drought and CO<sub>2</sub> enrichment

S

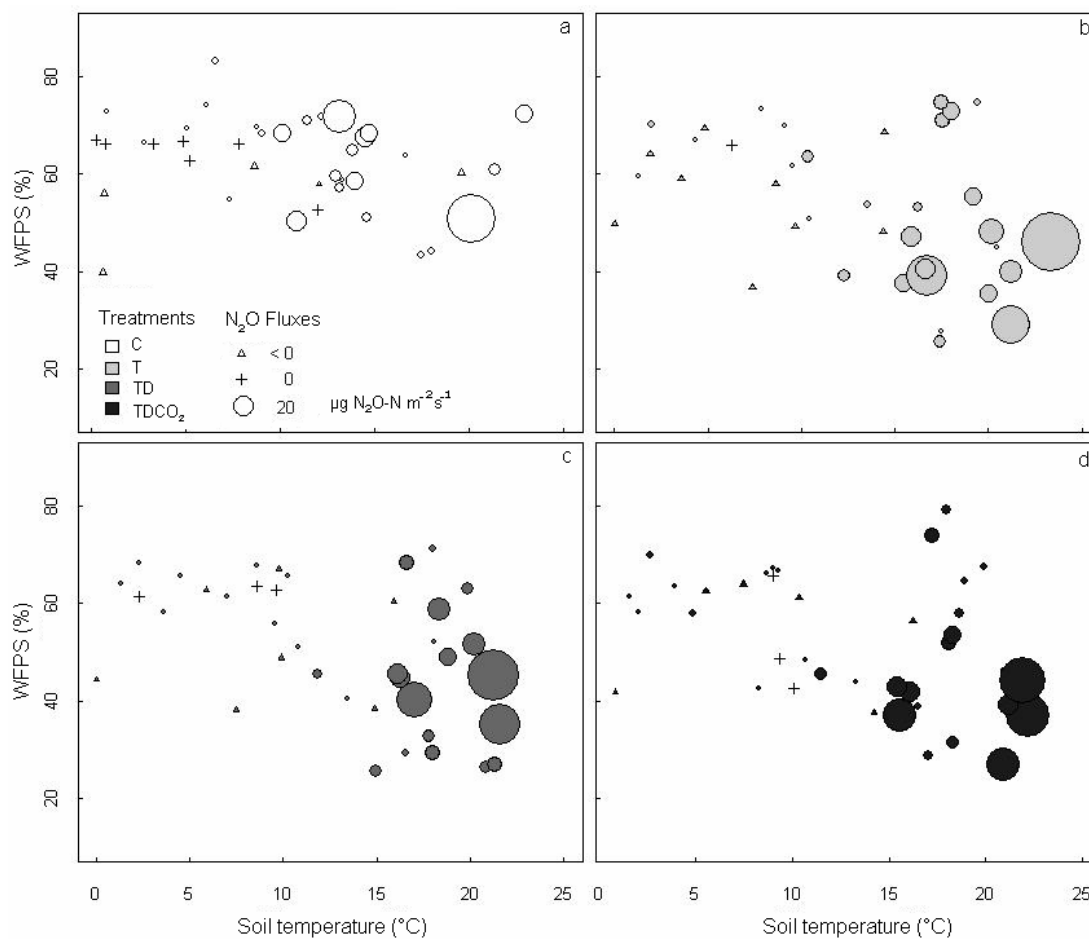


Figure 4. Effects of soil moisture (WFPS, %) and soil temperature (°C) on mean N<sub>2</sub>O fluxes under experimental climate treatments. Treatment codes are given by: C = control; T = elevated temperature; TD = elevated temperature and summer drought; TDCO<sub>2</sub> = elevated temperature, summer drought and CO<sub>2</sub> enrichment. . Sizes of symbols are proportional to the magnitude of N<sub>2</sub>O fluxes.

## 5- Discussion

### 5.1 N<sub>2</sub>O fluxes in extensively-managed grasslands

In this study we measured N<sub>2</sub>O fluxes in a pre-existing, long-term climate change experiment investigating the responses of an upland grassland subjected to single and combined climate change drivers applied in additive experimental design (Bloor *et al* 2010). Previous work has found little evidence for interactions among climate change drivers on biomass production, phenology and species composition in grassland ecosystems (Zavaleta *et al* 2003, Dukes *et al* 2005; Cleland *et al* 2006; Engel *et al* 2009). Consequently additive experimental designs provide a cost-efficient and effective approach to studying effects of climate scenarios with multiple factors on grasslands *in situ*. Of course we recognise that interactions between climate drivers may be more prevalent for N<sub>2</sub>O fluxes, and that a factorial design would allow these confounding effects to be tested. Nevertheless, the present study provides valuable information on grassland N<sub>2</sub>O fluxes in response to simultaneous climate changes.

Fluxes of N<sub>2</sub>O recorded at our control site were comparable with those reported in other studies of unfertilised, ungrazed grasslands (Yamulki *et al* 1997; Goossens *et al* 2001; Flécharde *et al* 2007; Kammann *et al* 2008). During the study period, we found maximum and minimum N<sub>2</sub>O fluxes of 73 and -13.1  $\mu\text{g N}_2\text{O-N m}^{-2} \text{h}^{-1}$  respectively in the control treatment. Peaks of N<sub>2</sub>O emissions were an order of magnitude smaller than those reported for fertilised grasslands with non-limiting soil N conditions (Flécharde *et al* 2007), consistent with the idea that N inputs are a key driver of N<sub>2</sub>O-N losses in managed grasslands.

Negative fluxes of N<sub>2</sub>O represented 6 % of the total number of measurements in the control treatment, and were mostly detected during winter-spring periods with low soil temperature (< 10 °C) and low WFPS (40-60 %). The net uptake of N<sub>2</sub>O is generally thought to arise from denitrification under conditions where nitrification is marginal, for example in the absence of NO<sub>3</sub><sup>-</sup> or in wet, poorly aerated soils (Goossens *et al* 2001; Chapuis-Lardy *et al* 2007). Occasional, small negative N<sub>2</sub>O fluxes have previously been demonstrated in the intervals between fertiliser applications (Ryden 1981; Clayton *et al* 1997) but the extent to which extensively-managed grassland soils can act as a sink for N<sub>2</sub>O remains unclear. Our data contrasts with that of Flecharde *et al* (2005), who found >40% occurrence of net N<sub>2</sub>O consumption in both wet and dry conditions at a Swiss grassland on calcareous soil (pH =

7.3). Discrepancies between the two studies could reflect differences in soil pH which modify nitrification activity (Wrage *et al* 2001); lower soil pH at our study site (pH = 6.2) should be more favourable for nitrification, and may explain the lower incidences of N<sub>2</sub>O consumption.

N<sub>2</sub>O fluxes at our upland grassland site showed considerable seasonal variation, with greater emissions in autumn and spring compared with winter fluxes. This pattern followed seasonal changes in soil temperature or soil moisture; increases in temperature and/or WFPS were associated with increased seasonal N<sub>2</sub>O emissions in agreement with previous studies (Clayton *et al* 1997; Almaraz *et al* 2009). Surprisingly, the combination of high temperatures and lower soil moisture (< 60 % WFPS) in summer did not result in a reduction in N<sub>2</sub>O emissions at the control site; mean summer N<sub>2</sub>O emissions were significantly greater than spring emissions despite a significant decrease in WFPS between seasons (-9 % and -6 % in 2007 and 2008 respectively, Appendix 2). These patterns suggest that temperature may have a relatively stronger influence on N<sub>2</sub>O fluxes between spring and summer. This finding is supported by multiple regression analysis of abiotic factors against N<sub>2</sub>O fluxes across measurement campaigns at the control site. As well as stimulating microbial processes related to N<sub>2</sub>O production, increased soil temperature may also facilitate N<sub>2</sub>O diffusivity in soils (Hernandez-Ramirez *et al* 2007).

Despite similar patterns of seasonal N<sub>2</sub>O emissions during the two-year study period, the magnitude of seasonal fluxes showed significant interannual variation. This interannual variation was due in part to variation in meteorological conditions between years; the summer period in 2008 was hotter on average than in 2007 (mean daily temperature of 17°C versus 15°C). In addition, we found a significant difference in average summer soil moisture conditions between years (61% versus 55% WFPS in 2008 and 2007 respectively Appendix 2) which must have favoured N<sub>2</sub>O emissions in 2008. These results highlight the importance of long-term studies for robust N<sub>2</sub>O inventories in grassland ecosystems.

## **5.2 N<sub>2</sub>O fluxes and climate change drivers**

Understanding the impacts of climate change on the magnitude of N<sub>2</sub>O emissions is critical for the estimation of N<sub>2</sub>O-N losses in grasslands under future climatic conditions. Irrespective of the choice of timeframe (measurement campaign, season, year), we found that N<sub>2</sub>O fluxes showed a stronger response to experimental warming alone than to summer drought under

warmed conditions or to elevated CO<sub>2</sub> combined with warming and summer drought. Elevated temperature had a positive effect on the magnitude of mean annual N<sub>2</sub>O emissions and on spring N<sub>2</sub>O fluxes in 2007. However, spring warming effects were not consistent between years, and we failed to detect any warming effects on N<sub>2</sub>O emissions in autumn despite favourable soil moisture conditions.

Limited warming effects on N<sub>2</sub>O fluxes observed in 2008 may partly reflect less pronounced differences in air and soil temperatures between control and warmed treatments during measurement campaigns in 2008 compared with 2007 (Table 1). Nevertheless, our results are in agreement with recent work in alpine Tibetan meadows, which report significant variation in the effects of warming on N<sub>2</sub>O fluxes depending on year, season, and sampling date (Hu *et al* 2010). How can we explain such variation in warming-induced N<sub>2</sub>O responses? Numerous studies provide evidence of threshold levels of soil moisture and temperature for microbial processes related to N<sub>2</sub>O production (De Klein & van Lotjstijn 1996; Bateman & Baggs 2005; Flechard *et al* 2007), which may promote temporal variability in warming effects. Warming effects on N<sub>2</sub>O fluxes may also be confounded by rainfall events since water-saturated soil surfaces promote the reduction of N<sub>2</sub>O to N<sub>2</sub> (Smith *et al* 2003). Finally, theoretical work suggests that local microbial parameters may play an important role in patterns of N<sub>2</sub>O fluxes (Henault *et al* 2005). The variable temperature effects observed in the present study support the idea that short-term grassland sensitivity to rises in temperature may vary depending on local biotic and abiotic conditions (Riedo *et al* 2000; Suh *et al* 2009).

Contrary to expectations, combined summer drought and warming had no significant effect on the frequency or magnitude of mean N<sub>2</sub>O fluxes recorded at any time. Decreases in soil moisture are often associated with a decrease in N<sub>2</sub>O fluxes (Dobbie & Smith 2001; Smith *et al* 2003), and negative effects of summer drought on N<sub>2</sub>O emissions have previously been demonstrated in forest ecosystems (Muhr *et al* 2008). Part of the explanation for our results may lie in the above-average summer rainfall experienced during the study period (+ 46.3 % and + 28.9 % for 2007 and 2008 respectively compared with the long-term average). Consequently, the impact of the experimental drought treatment on soil processes may have been attenuated by the ambient climate conditions at the control site. Of course it is possible that more pronounced drought effects may have been detected with greater numbers of measurement campaigns during the summer drought period; high spatial and temporal variability in N<sub>2</sub>O fluxes can mask short-term treatment effects (Flechard *et al* 2007).

Unlike soil moisture, elevated CO<sub>2</sub> has been shown to have mixed effects on mean N<sub>2</sub>O fluxes in grassland studies (Baggs *et al* 2003; Kettunen *et al* 2007; Kammann *et al* 2008). The lack of CO<sub>2</sub> response observed in our unfertilised study system under warmed conditions with summer drought may reflect N-limited microbial populations with limited potential for increased activity in response to CO<sub>2</sub>-induced C inputs (Hu *et al* 2005), or modified competition for soil N between plants and microbial communities under elevated CO<sub>2</sub> (Loiseau & Soussana 2000; Bloor *et al* 2009). Although mean N<sub>2</sub>O fluxes were insensitive to elevated CO<sub>2</sub> in the present study, it is important to note that the greatest peaks of N<sub>2</sub>O emissions for individual grassland monoliths were recorded in the TDCO<sub>2</sub> treatment (maximum 203 µg N<sub>2</sub>O-N m<sup>-2</sup> h<sup>-1</sup>). Such N<sub>2</sub>O emissions are comparable to peaks of emissions documented in fertilised grasslands (Kammann *et al* 1998), and suggest that elevated CO<sub>2</sub> could promote transient but important N losses in response to rewetting even in extensively-managed grasslands. Peaks in N<sub>2</sub>O emission under simultaneous warming, summer drought and elevated CO<sub>2</sub> could be enhanced by CO<sub>2</sub>-induced increases in mineralization rates, which provide a supplementary N source for denitrification (Zak *et al* 2000). Automated N<sub>2</sub>O measurements with a daily time resolution are required to improve our understanding of the interactions between climate drivers, soil moisture and re-wetting events on N<sub>2</sub>O emissions in future climatic scenarios.

Previous work on European grasslands has shown that much of the variation in fertiliser-derived N<sub>2</sub>O emissions (i.e. emission factors) across sites can be predicted on the basis of soil temperature, moisture and rainfall (Flechard *et al* 2007). In our study, these three parameters only explained 32 - 48 % of the variation in mean N<sub>2</sub>O fluxes recorded in the experimental climate change treatments. Nevertheless, we found some evidence for treatment-driven changes in the relationship between N<sub>2</sub>O fluxes and abiotic factors. Our data suggest not only that the relative importance of soil temperature on N<sub>2</sub>O fluxes is reduced under experimental warming, but also that elevated CO<sub>2</sub> may reduce the dependency of N<sub>2</sub>O fluxes on rainfall inputs. Further work is required to disentangle direct and indirect effects of climate change drivers on N<sub>2</sub>O-N losses.

## 6. Conclusions

N<sub>2</sub>O fluxes showed significant seasonal and interannual variation in our extensively-managed, upland grassland system. Overall, experimental warming had a positive effect on the annual

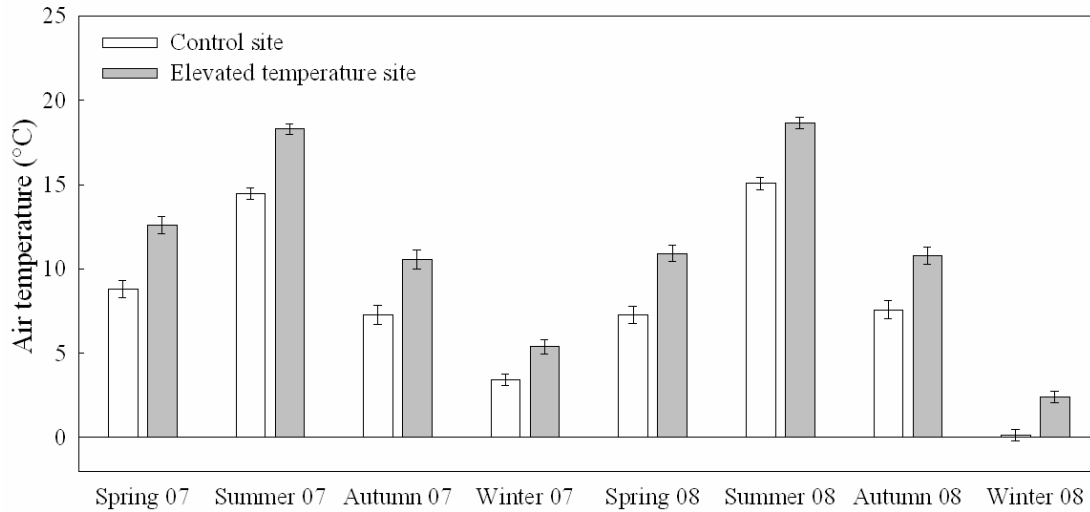
N<sub>2</sub>O emissions, strongly driven by 2007. In contrast, neither summer drought under experimental warming nor elevated CO<sub>2</sub> in combination with warming and drought had significant effects on N<sub>2</sub>O fluxes in elevated temperature conditions. Our data suggests that under projected future climate conditions, N<sub>2</sub>O fluxes in cool, upland grasslands may be driven primarily by responses to elevated temperature. Additional work is needed to examine the relative importance of microbial community structure and function on patterns of N<sub>2</sub>O fluxes under future climate conditions. Longer-term, automated N<sub>2</sub>O measurements with a higher sampling frequency should also determine how soil moisture modulates the impact of climate drivers on N<sub>2</sub>O emissions.

## **7. Acknowledgements**

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## 8. Appendix

### Appendix 1



*Appendix 1. Seasonal averages of daily air temperature recorded during the present study for the control site compared with the elevated temperature site. Means and standard errors are presented (n = 90).*

### Appendix 2

*Appendix 2. Seasonal averages of soil moisture (WFPS, %) recorded during the N<sub>2</sub>O campaign measurements for experimental climate treatments. Means and standard errors are presented (n = 20-30).*

	Climate treatment			
	C	T	TD	TDCO2
<i>a) 2007</i>				
Winter	66.1 ± 0.5	65.7 ± 1.5	60.9 ± 0.9	62.4 ± 1.5
Spring	61.0 ± 0.7	61.7 ± 2.1	56.1 ± 2.0	51.5 ± 1.7
Summer	55.3 ± 1.6	52.4 ± 1.7	30.2 ± 1.3	39.1 ± 0.8
Autumn	62.1 ± 2.8	60.9 ± 2.1	47.0 ± 1.6	49.3 ± 1.7
<i>b) 2008</i>				
Winter	61.3 ± 3.3	61.7 ± 1.8	61.5 ± 0.6	60.2 ± 1.8
Spring	65.2 ± 0.8	64.2 ± 1.9	60.6 ± 1.2	55.3 ± 2.6
Summer	60.7 ± 2.1	57.7 ± 3.9	49.9 ± 3.2	46.1 ± 3.6
Autumn	67.9 ± 2.8	58.9 ± 3.1	59.7 ± 0.8	51.7 ± 3.0



## **Résultats - Chapitre 4**

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**Le changement climatique affecte la dynamique et les flux d'oxyde nitreux (N<sub>2</sub>O) en prairies, via des changements des activités enzymatiques et des pools de gènes des communautés microbiennes à l'origine des flux.**

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Les résultats du chapitre 3 posent la question de l'importance du fonctionnement et de la structure de l'écosystème microbien à l'origine des flux de N<sub>2</sub>O sous changement climatique. Ce chapitre cherche à mettre en évidence les processus et mécanismes microbiens à l'origine des flux de N<sub>2</sub>O en prairie sous l'influence d'un changement climatique. Il est présenté sous forme d'un article en préparation pour la revue '*The ISME Journal*'. Il s'intitule: 'Climate change drivers modify the response potential of microbial enzymatic activity and microbial abundances on N<sub>2</sub>O fluxes in a grassland experiment.' Les co-auteurs sont Franck Poly<sup>a</sup>, Juliette Bloor<sup>b</sup>, Nadine Guillaumaud<sup>a</sup>, Caroline Moiro<sup>a</sup> et Jean-François Soussana<sup>c</sup>.

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a : Laboratoire d'Ecologie Microbienne, Université Lyon1, France

b : INRA - UR 874 Clermont-Ferrand, France

c : INRA - CODIR - ENVIRONNEMENT Paris, France

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## 1- Abstract

Emissions of the trace gas nitrous oxide (N<sub>2</sub>O) play an important role for the greenhouse effect and stratospheric ozone depletion, but the impact of climate change on N<sub>2</sub>O fluxes and the underlying microbial drivers is unclear. The aim of this study was to determine effects of climate change on field N<sub>2</sub>O fluxes, associated microbial processes (nitrification and denitrification) and microbial population size (AOB, *nirK* and *nosZ*) in an extensively managed, upland grassland. Over the course of one year, we recorded N<sub>2</sub>O fluxes, microbial abundance and enzymatic activities in a grassland exposed to elevated atmospheric CO<sub>2</sub> (+200 ppm), elevated temperature (+3.5°C) and reduction of summer precipitations (-20%) as part of a long-term, multifactor climate change experiment. Our results showed that: 1) both warming and simultaneous application of warming, summer drought and elevated CO<sub>2</sub> had a positive effect on N<sub>2</sub>O fluxes, nitrification, N<sub>2</sub>O release by denitrification and the population size of *nosZ* and AOB genes; 2) the magnitude of climate-induced changes on measured variables could be ranked: N<sub>2</sub>O fluxes > NEA > N<sub>2</sub>O release by denitrification (N<sub>2</sub>O<sub>DEA</sub>) > *nosZ* pool > AOB pool > *nirK* pool and N<sub>2</sub> release by denitrification (N<sub>2</sub>DEA); 3) irrespective of climate change treatment, variation in field N<sub>2</sub>O emissions showed a positive correlation with denitrification ratio (N<sub>2</sub>O<sub>DEA</sub>/[N<sub>2</sub>O<sub>DEA</sub>+ N<sub>2</sub>DEA]); 4) field N<sub>2</sub>O fluxes showed a stronger correlation with microbial population size under warmed conditions compared with the unwarmed control site.



## 2- Introduction

In recent decades, changes in land use and human activities have had significant impacts on gaseous nitrogen (N) losses and the global cycle of N, contributing to regional and global changes in the atmosphere (IPCC 2007). Emissions of nitrous oxide (N<sub>2</sub>O) are of particular interest because this trace gas has a strong global warming potential (~320 times greater than that of carbon dioxide) and is the single most important ozone-depleting emission (Ravishankara *et al.* 2009). The magnitude of N<sub>2</sub>O emissions depends on both microbial activities (ammonia oxidizing bacteria: AOB and/or denitrifiers, Bremner 1997; Wrage *et al.* 2004) and abiotic factors including soil temperature, oxygenation, mineral nitrogen, pH and water content (Simek *et al.* 2002; Smith *et al.* 2003; Jones *et al.* 2005). Consequently, understanding the interplay between microbial and environmental variables is critical for the estimation of potential N<sub>2</sub>O fluxes from soils under climate change.

Despite a large number of studies documenting gaseous N<sub>2</sub>O emissions from grassland ecosystems, few have focused on impacts of climate change drivers on N<sub>2</sub>O fluxes and associated microbial processes (Clayton *et al.* 1997, Flécharde *et al.* 2005, Flécharde *et al.* 2007; but see Avrahami & Bohannan 2009). In theory, warming is expected to have positive effects on nitrification and denitrification rates (Godde & Conrad, 1999), with cascading effects on N<sub>2</sub>O emissions. However, warming responses of both nitrification and denitrification have been found to be highly variable across studies (Shaw & Harte 2001; Emmett *et al.* 2004; Horz *et al.* 2004; Hart 2006; Malchair *et al.* 2010; Szukics *et al.* 2010), reflecting in part, variable soil water content status during experiments (Barnard & Leadley 2005). Impacts of reduced soil moisture status on microbial processes are well established (Rustad, 2001, Barnard & Leadley 2005; Barnard *et al.* 2005; Bateman & Baggs 2005), and determine the balance between nitrification and denitrification via changes in soil aeration and O<sub>2</sub> content (Smith *et al.* 2003). In addition, elevated CO<sub>2</sub> may alter microbial processes by both increasing soil moisture (Smith & Tiedje 1979) and carbon substrate availability (Luo & Mooney 1999). Previous work suggests that elevated CO<sub>2</sub> may have greater effects on denitrification than nitrification (Baggs *et al.* 2003, Barnard *et al.* 2004, Barnard *et al.* 2006), but considerable variation is observed across studies.

Whilst information on N<sub>2</sub>O emissions and microbial activities subjected to individual climate change drivers is becoming increasingly available (Barnard *et al.* 2004; Barnard *et al.* 2006; Bateman & Baggs 2005; Kamman *et al.* 2008; Hu *et al.* 2010; Malchair *et al.* 2010), data on N<sub>2</sub>O flux responses to multiple and simultaneous environmental changes are scarce. In a recent study examining the impact of co-occurring climatic changes on N<sub>2</sub>O fluxes in an upland grassland, Cantarel *et al.* (2011) found that N<sub>2</sub>O fluxes responded more strongly to warming alone than to the combination of summer drought or elevated CO<sub>2</sub> and warmed conditions. Results from laboratory incubations suggest that interactions between soil moisture and temperature can generate complex patterns of N<sub>2</sub>O emissions under controlled conditions (Avrahami & Bohannan 2009), but the importance of multiple climate changes for field N<sub>2</sub>O emissions remains unclear.

In addition to direct climate-induced changes in microbial activities, climate change drivers can impact N transformations and N<sub>2</sub>O emissions via indirect effects on the abundance of different microbial populations, and microbial community structure. Variation in soil N<sub>2</sub>O emissions may reflect differences in the abundances and/or diversity of AOB (Ammonium oxidizing archaea are not involved in N<sub>2</sub>O emission, Di *et al.* 2010) and denitrifying microorganisms (Philippot *et al.* 2010, Avrahami & Bohannan 2009). To date, only AOB community structure has been studied for grasslands subjected to complex, multiple climate change treatments (Horz *et al.* 2004). Horz and coworkers found that abundance of AOB decreased in response to combined elevated CO<sub>2</sub> and increased precipitation, but these effects appeared to be buffered under elevated temperature conditions. The potential impact of multiple climatic variables on the microbial community structure, and respective contributions of AOB and denitrifying microorganisms to N<sub>2</sub>O fluxes remains to be confirmed for other grassland ecosystems.

Here we examine the relationship between field N<sub>2</sub>O fluxes and soil microbial communities under three key climate change drivers in an upland grassland ecosystem. Measurements were carried out at the Clermont Climate Change Experiment facility (Bloor *et al.*, 2010), a long-term climate change study manipulating air temperature ( $\pm 3.5$  °C), atmospheric CO<sub>2</sub> ( $\pm 200$  ppm) and summer drought ( $\pm 20$  % summer rainfall) in an additive experimental design. The aims of the study were to evaluate seasonal variation in N<sub>2</sub>O fluxes, nitrifying and denitrifying enzyme activities, and abundance of microorganisms (AOB, nirK and nosZ estimated by qPCR targeting functional microbial groups) under single and

combined climate change treatments. Specifically we ask: (1) How do nitrification, denitrification and abundance of microbial nitrifiers/ denitrifiers respond to climate change treatments? ; (2) Is variation in field N<sub>2</sub>O fluxes mirrored by changes in microbial activities or abundance of specific microbial populations?

### **3- Materials and methods**

#### ***3.1 Experimental design and climate treatments***

The study system is an upland permanent grassland in the French Massif Central region (45°43'N, 03°01'E, 850 m a.s.l.), characterised by a Cambisol soil (59.5% sand, 19.7% silt, 20.8% clay, pH 6.2), and a grass-dominated plant community (*Festuca arundinaceae*, *Elytrigia repens*, *Poa pratensis*). The study area has a mean annual temperature of 8.7 °C and a mean annual rainfall of 780 mm.

The Clermont Climate Change Experiment was established in 2005, manipulating air temperature, summer rainfall and atmospheric CO<sub>2</sub> in line with IPCC projections for the study area in 2080 (ACACIA A2 scenario, IPCC 2001; see Bloor *et al.* 2010 for full details). In brief, the experiment consists of 80 grassland monoliths (0.5 x 0.5 x 0.4 m in size), excavated from the study grassland site and allocated at random to one of four climate treatments; C (control), T (+3.5°C), TD (+3.5°C, 20% reduction in summer rainfall) and TDCO<sub>2</sub> (+3.5°C, 20% reduction in summer rainfall, CO<sub>2</sub> levels of 600ppm). Each experimental treatment comprises of five experimental units (or repetitions), formed by grouping four monoliths together in specially prepared cavities in the ground. Elevated temperatures were achieved by transporting monoliths to a nearby lower-altitude site (Clermont-Ferrand, 350m a.s.l.). Summer drought was established by the use of rain screens and modified watering regimes during June, July and August. Enrichment of atmospheric CO<sub>2</sub> was obtained by using Mini-FACE (Free Air Carbon dioxide Enrichment) technology; the target CO<sub>2</sub> concentration was operational during daylight hours.

Meteorological measurements were made using a Campbell Scientific automatic weather station and logged to a CRX-10 data logger (Campbell scientific Inc., Utah, USA) at 30 minute intervals for both the upland and lowland sites. Volumetric soil moisture (0-20 cm)

was recorded hourly using ECH<sub>2</sub>O-20 probes (Dielectric Aquameter, Decagon Devices, Inc., Pullman, WA). Vegetation in all experimental units was cut to a height of 5 cm at six-month intervals (April and October). Monoliths were left to grow without the addition of fertiliser throughout the study period.

### **3.2 N<sub>2</sub>O flux measurements and soil sampling**

N<sub>2</sub>O fluxes were determined every two months between May and November 2009, using medium-size, closed and non-vented manual chambers on one monolith per experimental unit (see Cantarel *et al.* 2011 for full details). During each N<sub>2</sub>O measurement campaign, chambers were fixed onto a permanent base for each target monolith and gas samples were taken at five twenty-minute intervals using a quick release pneumatic connector (TST Tansam Inc, Kocaeli, Turkey) and a PTFE-Teflon tube connected to an INNOVA 1412 photoacoustic multi-gas analyser (INNOVA AIR Tech Instruments, Ballerup, Denmark). The INNOVA gas analyser was encased in an air- conditioned box to avoid confounding effects of temperature on analyser measurements. N<sub>2</sub>O fluxes were calculated by linear regression; flux data were rejected if the statistic p-value was below 0.05. Soil temperature in the topsoil layer (2-5cm) was recorded by thermocouples (TC S.A., Dardilly, France) during N<sub>2</sub>O measurement campaigns. Field N<sub>2</sub>O fluxes were measured during warm conditions (29 May, 27 July) and during colder conditions (23 September and 28 November). Immediately following field N<sub>2</sub>O measurements, 3 soil cores (diameter 1.5 cm) were taken from the top soil (0-10cm) of each target monolith, pooled together and sieved at 4 mm. Soils were stored for less than five days at 4°C before carrying out assays for nitrification and denitrification enzyme activity (NEA, DEA) (see below). A subsample of fresh soil was frozen at -18°C for molecular analyses.

### **3.3 Denitrifying and nitrifying enzyme activities**

Denitrification enzyme activity (DEA) was measured in fresh soils from each monolith following Patra *et al.* (2006). Two sub-samples (10g equivalent dry soil) from each soil sample were placed into 150ml plasma flasks, and 7ml of solution containing KNO<sub>3</sub> (50 µg NO<sub>3</sub><sup>-</sup> N g<sup>-1</sup> dry soil), glucose (0.5 mg C g<sup>-1</sup> dry soil) and glutamic acid (0.5mg C g<sup>-1</sup> dry soil) were added. Additional distilled water was provided to achieve 100% water holding capacity. The atmosphere was replaced by He to provide anaerobic conditions and for one flask of each



pair, 10% C<sub>2</sub>H<sub>2</sub> was added to inhibit N<sub>2</sub>O reductase activity with C<sub>2</sub>H<sub>2</sub>. During incubation at 28°C, gas samples were taken at 2h, 3h30, 5h, 6h30 and immediately analysed for N<sub>2</sub>O using a gas chromatograph (R3000µGC, SRA instrument, Marcy l'Etoile, France). For the first samples (without C<sub>2</sub>H<sub>2</sub>), we measured N<sub>2</sub>O accumulation, i.e. potential N<sub>2</sub>O emission rates of our soil, or N<sub>2</sub>O<sub>DEA</sub>. Samples with C<sub>2</sub>H<sub>2</sub> allowed determination of maximal N<sub>2</sub>O production or N<sub>2</sub>O<sub>TOT</sub>. We estimated potential fluxes of N<sub>2</sub> (or N<sub>2</sub>DEA) by subtraction of N<sub>2</sub>O<sub>DEA</sub> from N<sub>2</sub>O<sub>TOT</sub>.

Nitrification enzyme activity (NEA) was determined following (Dassonville *et al.* in press). In brief, sub-samples of fresh soil (3 g equivalent dry soil) were incubated with 6 ml of a solution of N-NH<sub>4</sub> (50 µg N-(NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> g<sup>-1</sup> dry soil). Distilled water was adjusted in each sample to achieve 24 ml of total water in flasks. The flasks were sealed with parafilm © and incubated at 28°C with constant agitation (180 rpm). During incubation, 1.5 ml of soil slurry was sampled at 1h, 2h30, 4h, 5h30 and 7h, filtered (0.2 µm pore size) and then analysed for NO<sub>2</sub><sup>-</sup> / NO<sub>3</sub><sup>-</sup> concentrations on an ionic chromatograph (DX120 Dionex, Salt Lake City, USA). A linear rate of NO<sub>2</sub><sup>-</sup> + NO<sub>3</sub><sup>-</sup> production was always observed (NEA). The intercept was used to estimate pools of soil NO<sub>3</sub><sup>-</sup> (NO<sub>3</sub><sup>-</sup>).

### **3.4 Soil DNA extraction and quantification of AOB, nirK and nosZ abundances**

DNA was extracted for each frozen soil subsample (0.5 g equivalent dry soil) using the 96 Well Soil DNA Isolation Kit (MO BIO laboratories, Carlsbad, CA, USA) and manufacturer protocols. The quantity of the DNA extraction was checked using the Quant-iT<sup>TM</sup> PicoGreen® method (Quant-iT<sup>TM</sup> PicoGreen® dsDNA Assay kit, Molecular Probes Inc., Eugene, OR, USA).

All gene quantifications were obtained by qPCR, using a Lightcycler 480 (Roche Diagnostics, Meylan, France). The abundance of nirK genes was determined by using SYBR Green as the detection system in a reaction mixture of 20µl, with 10 µl of SYBR Green PCR master mix, including HotStar Tap<sup>TM</sup> DNA polymerase, QuantiTec SYBR Green PCR buffer, dNTP mix with dUTP SYBR Green I, ROX and 5 mM MgCl<sub>2</sub> (QuantiTect<sup>TM</sup> SYBR® Green PACR Kit, QIAGEN, France), 1 µM of nirK876 primer (ATYGGCGGVAYGGCGA), 1 µM of nirK1040 primer (GCCTCGATCAGRTTRTGGTT) (Henry *et al.* 2004), 0.4 µg of T4 gene protein 32 (QBiogene, France), 5 ng of soil DNA and Rnase-free water to complete the 20 µl volume. The conditions for nirK qPCR were 15 min at 95°C for denaturation; 45 cycles at

95°C for 15 s, 63°C for 30 s and 72°C for 30 s for amplification ; 1s at 95°C and 20 s at 68°C for acquisition step and 10 s at 40°C to finish analysis.

The abundance of  $\beta$ -proteobacterial AOB, that represents known AOB in soil, and which are potentially implied in N<sub>2</sub>O emissions (Wrage *et al.* 2004) (contrarily to AOA never shown to product N<sub>2</sub>O) was measured by qPCR targeting 16S rRNA gene sequences specific for this group (Hermansson & Lindgren, 2001). The final reaction volume was 20  $\mu$ l, with 0.5  $\mu$ M of a 2:1 ratio of primer CTO189fA/B and CTO189fC, 0.5  $\mu$ M of RT1r primer, 0.5  $\mu$ M of TPM1probe, 0.4 mg ml<sup>-1</sup> bovine serum albumin (BSA), 10 ng of sample DNA or standard DNA with number of copies known. The samples were run as follows: 10 min at 95°C; 45 cycles at 95°C for 10 s, 58°C for 20 s, and 1 s at 72°C; and 30 s at 40°C.

For *nosZ* gene quantification, primers nosZ2F (5' CGCRACGGCAASAAGGTSMSSGT 3') and nosZ2R (5' CAKRTGCAKSGCRTGGCAGAA 3') (Henry *et al.* 2006) were used. The final volume 25 $\mu$ L PCR mix contained: QuantitTect SybrGreen PCR Master Mix 1X (Qiagen, Courtaboeuf, France), 0.1 $\mu$ g of T4 gene protein 32 (QBiogene, France), 1 $\mu$ M of each primer, and 5 ng of soil DNA extract or 5 $\mu$ L of tenfold standard serial dilution ranging from 10<sup>7</sup> to 10<sup>2</sup> *nosZ* copies of genomic DNA from *Pseudomonas aeruginosa* PA14. Thermal cycling was carried out by using an initial enzyme activation step at 95°C for 10 min followed by 55 cycles of denaturation at 95°C for 15 s, annealing at 68°C for 30sec with a touchdown of -1°C by cycle until reach 63°C and elongation at 72°C for 30s.

### 3.5 Statistical analyses

Effects of climate treatment on N<sub>2</sub>O, NEA, NO<sub>3</sub><sup>-</sup>, N<sub>2</sub>O<sub>DEA</sub>, N<sub>2</sub>DEA, and abundance of gene copies (AOB, nirK, *nosZ*) were analysed using mixed model repeated measures analysis of variance (ANOVA) with both treatment and date as fixed factors (Zar 1998). Effects of individual climate change drivers (temperature, drought, CO<sub>2</sub>) were analysed using orthogonal contrasts (Gilligan 1986). Effects of warming were determined by comparing the C and T treatment; effects of summer drought by comparing T and TD; effects of elevated [CO<sub>2</sub>] by comparing TD and TDCO<sub>2</sub>; effects of simultaneous application of warming, summer drought, and CO<sub>2</sub> enrichment (2080 climate scenario) were investigated by the C versus TDCO<sub>2</sub> comparison. Relationships between field N<sub>2</sub>O fluxes, potential activities and

gene abundances were examined using Spearman correlation coefficients. All analysis was carried out using Statgraphics Plus 4.1 (Statistical Graphics Corp., Rockville, Maryland, USA).

## 4- Results

### 4.1 Climate

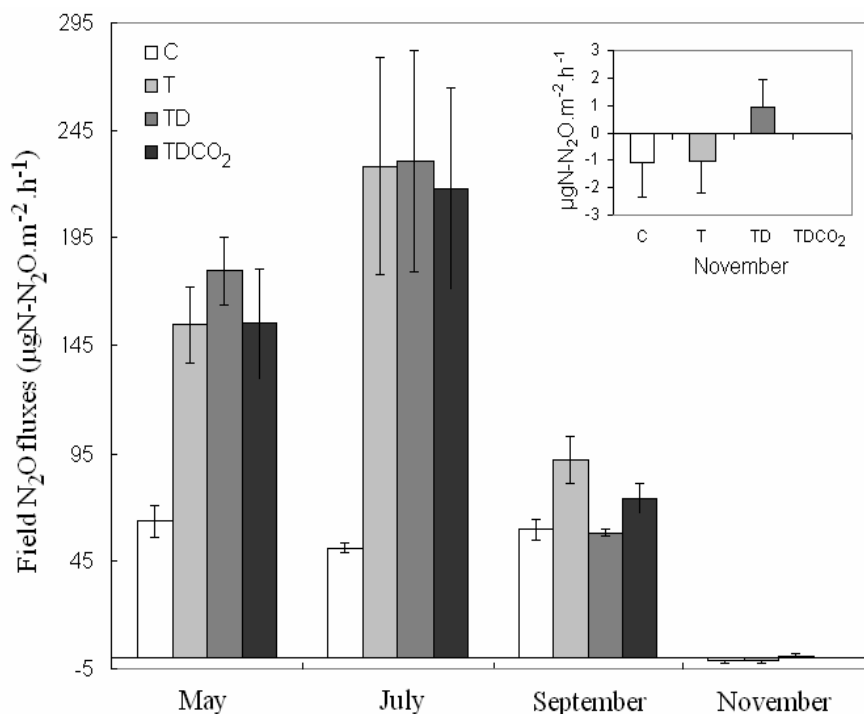
From May 2009 to November 2009, the difference in mean monthly temperature between control and elevated temperature treatments was  $3.4 \pm 0.03$  °C. Differences in precipitation between C and T treatments ( $18.2 \pm 0.9$  mm) represented, < 4 % of total rainfall during this period. In summer (June, July, August) 2009, the warmed, drought (TD, TDCO<sub>2</sub>) treatments received a 21 % reduction in rainfall compared with the T treatment. Mean daily CO<sub>2</sub> differences between the TDCO<sub>2</sub> treatment and the ambient CO<sub>2</sub> treatments (C and T) were  $193.3 \pm 13.1$  ppm. Microclimatic variables recorded on days of N<sub>2</sub>O measurement indicated no differences in soil moisture between the C, T and TDCO<sub>2</sub> treatments for the four sampling dates (Table 1). The TD treatment had drier soil moisture conditions than the T treatment in July, September. On average, the warmed treatments (T, TD and TDCO<sub>2</sub>) had higher soil temperature compared with the control treatment (C). Soil temperature and WFPS showed a significant regression against time, soil temperature appearing to progressively decrease over time ( $r^2 = 67.4$ ,  $P < 0.001$ ) and WFPS to progressively increase over time ( $r^2 = 73.9$ ,  $P < 0.001$ ).

**Table 1: Mean soil moisture (WFPS, %) and soil temperature recorded during each N<sub>2</sub>O measurement dates for experimental climate treatments. Means and standard errors are presented (n = 5).**

		May	July	September	November
WFPS (%)	C	31.9 ± 0.0	35.9 ± 0.0	50.1 ± 0.0	52.9 ± 0.3
	T	29.3 ± 1.1	32.7 ± 1.6	45.2 ± 2.9	52.6 ± 2.3
	TD	32.4 ± 0.8	29.4 ± 0.3	30.6 ± 0.3	48.8 ± 0.2
	TDCO <sub>2</sub>	29.5 ± 0.8	37.3 ± 1.8	47.1 ± 1.7	51.8 ± 1.5
Soil temperature (°C)	C	17.3 ± 0.4	17.3 ± 0.4	14.3 ± 0.2	4.2 ± 0.3
	T	22.7 ± 1.5	23.8 ± 0.6	17.1 ± 0.5	6.3 ± 0.6
	TD	23.5 ± 1.2	24.9 ± 1.1	17.3 ± 0.7	5.7 ± 0.7
	TDCO <sub>2</sub>	22.6 ± 1.2	22.9 ± 0.9	17.4 ± 0.8	6.7 ± 0.6

#### 4.2 Effects of climate change drivers on N<sub>2</sub>O fluxes in situ

Over the course of the experiment, N<sub>2</sub>O fluxes ranged from -5.5 to 369.2 µg N<sub>2</sub>O-N m<sup>-2</sup> hr<sup>-1</sup> across treatments (Figure 1). N<sub>2</sub>O fluxes showed significant effects of treatment and date (repeated measures ANOVA,  $F_{1,3} = 6.6$ ,  $P < 0.001$  and  $F_{1,3} = 31.7$ ,  $P < 0.001$  respectively) but effects of climate drivers varied over time (significant Treatment x Date interaction;  $F_{1,9} = 2.16$ ,  $P < 0.05$ ; Figures 1). The significant interaction was driven by very low N<sub>2</sub>O fluxes in November across all climate treatments. With the exception of the November sampling date, warming (C versus T comparison) had a positive effect on N<sub>2</sub>O emissions ( $F_{1,16} = 23.1$ ,  $P < 0.001$ ;  $F_{1,16} = 6.6$ ,  $P < 0.05$  and  $F_{1,16} = 14.6$ ,  $P < 0.01$  respectively for May, July and September). This pattern of response was also found for the combined climate change drivers (C versus TDCO<sub>2</sub>) in May and July. Unlike warming and combined climate change, summer drought (T versus TD) and elevated CO<sub>2</sub> (TD versus TDCO<sub>2</sub>) had little impact on N<sub>2</sub>O fluxes. However, drought was associated with a significant negative effect on N<sub>2</sub>O fluxes in September ( $F_{1,16} = 15.5$ ,  $P < 0.01$ ; Figure 1).



**Figure 1: Effects of climate manipulations on N<sub>2</sub>O fluxes for measurement dates in spring, summer and autumn 2009. Treatments are given by: C = Control; T = elevated temperature (+3.5°C); TD = elevated temperature and summer drought (+3.5 °C, -20 %); TDCO<sub>2</sub> = elevated temperature, summer drought and CO<sub>2</sub> enrichment (+3.5 °C, -20 %, +200 ppm). Means and standard errors are presented per treatment and measurement date (n = 5).**

### 4.3 Changes in nitrifying and denitrifying enzyme activities

Over the study period, NEA showed significant effects of treatment (repeated measures ANOVA,  $F_{1,3} = 5.1$ ,  $P < 0.01$ , Table 2). Irrespective of measurement date examined, warming and combined climate treatments had a positive impact on NEA ( $F_{1,16} = 5.7$ ,  $P < 0.05$  and  $F_{1,16} = 6.9$ ,  $P < 0.05$  respectively). Both climate treatment and date had a significant effect on nitrate pools and N<sub>2</sub>O<sub>DEA</sub>, but treatment effects were consistent between dates (no significant Treatment x Date interaction). Warming and the simultaneous application of warming, drought and CO<sub>2</sub> treatments had a positive effect on NO<sub>3</sub><sup>-</sup> ( $F_{1,38} = 10.67$ ,  $P < 0.01$  and  $F_{1,38} = 10.85$ ,  $P < 0.01$  for C versus T and C versus TDCO<sub>2</sub> respectively, Table 2). In addition combined warming, drought and elevated CO<sub>2</sub> had a positive effect on N<sub>2</sub>O<sub>DEA</sub> across all measurement dates ( $F_{1,38} = 5.4$ ,  $P < 0.05$ ). In contrast, N<sub>2</sub>DEA and denitrification product ratio (N<sub>2</sub>O<sub>DEA</sub>/[N<sub>2</sub>O<sub>DEA</sub>+ N<sub>2</sub>DEA]) showed no response to climate treatments. Neither summer drought under warmed conditions (T versus TD) nor elevated CO<sub>2</sub> in combination with warming and summer drought (TD versus TDCO<sub>2</sub>) had any significant effect on nitrifying and denitrifying enzyme activities. Across treatments, NO<sub>3</sub><sup>-</sup>, N<sub>2</sub>O<sub>DEA</sub>, N<sub>2</sub>DEA and denitrification product ratio showed a significant effect of measurement date (Table 2). NO<sub>3</sub><sup>-</sup> and N<sub>2</sub>DEA showed a progressive increase over time ( $r^2 = 37.7$ ,  $P < 0.001$  and  $r^2 = 23.8$ ,  $P < 0.001$  respectively), whereas N<sub>2</sub>O<sub>DEA</sub> and denitrification product ratio showed a progressive decrease over time ( $r^2 = 18.3$ ,  $P < 0.001$  and  $r^2 = 49.6$ ,  $P < 0.001$  respectively).

### 4.4 Changes in the abundances of AOB, nirK and nosZ

Both climate treatment and dates had a significant effect on abundance of the AOB gene ( $F_{1,3} = 2.3$ ,  $0.1 < P < 0.05$ , and  $F_{1,3} = 15.5$ ,  $P < 0.001$  respectively; Table 3), but effects of treatment varied depending on date (significant Treatment x Date interaction,  $F_{1,9} = 2.4$ ,  $P < 0.05$ ). In general, numbers of AOB copies were higher in November compared to the dates in spring and summer. Warming had a positive effect on the abundance of AOB in May and November ( $F_{1,16} = 6.3$ ,  $P < 0.05$  and  $F_{1,16} = 9.1$ ,  $P < 0.01$  respectively), whereas combined climate and elevated CO<sub>2</sub> alone was only associated with an increase in AOB in November ( $F_{1,16} = 8.5$ ,  $P < 0.05$  for combined climate and  $F_{1,16} = 16.5$ ,  $P < 0.001$  for elevated CO<sub>2</sub>). Abundance of *nirK* genes showed no significant response to climate treatments over the four measurement dates (Table 3). However, the number of *nosZ* copies showed a significant response to both

treatment and measurement date (Table 3). Effects of treatment did not differ significantly between dates (no significant Treatment x Date interaction), and both experimental warming and combined climate change were found to have a positive effect on nosZ abundance. Furthermore, a progressive increase of nosZ gene abundance was found over time ( $r^2 = 12.5$ ,  $P < 0.001$ ).

**Table 2: Effects of climate change treatment on (a) NO<sub>3</sub><sup>-</sup>, (b) NEA, (c) N<sub>2</sub>O<sub>DEA</sub>, (d) N<sub>2</sub>O<sub>DEA</sub> and (e) denitrification product ratio (means and standard errors are shown; n = 5). Results from repeated measures ANOVA testing the effects of climate change treatments, measurement dates and their interaction are presented (significant P values are shown in bold).**

	Climate treatments				Repeated measures ANOVA			
	C	T	TD	TDCO <sub>2</sub>	p-model	Treatments	Dates	Treatments x Dates
<i>a) NO<sub>3</sub><sup>-</sup> (μg N-NO<sub>3</sub><sup>-</sup>.g<sup>-1</sup>)</i>								
May	3.1 ± 0.5	2.8 ± 0.6	2.5 ± 0.6	3.2 ± 0.6	***	<b>0.002</b>	<b>&lt;0.001</b>	0.080
July	4.3 ± 0.4	5.5 ± 0.9	6.2 ± 1.9	7.2 ± 0.8				
September	6.8 ± 1.1	14.7 ± 3.9	15.5 ± 3.2	10.3 ± 2.3				
November	5.2 ± 0.8	13.3 ± 2.8	14.3 ± 3.6	14.9 ± 2.9				
<i>b) NEA (μg N(NO<sub>2</sub>+NO<sub>3</sub>).g<sup>-1</sup>.h<sup>-1</sup>)</i>								
May	0.51 ± 0.09	0.82 ± 0.09	0.87 ± 0.16	0.89 ± 0.16	*	<b>0.003</b>	0.238	0.807
July	0.53 ± 0.06	0.70 ± 0.16	0.89 ± 0.14	0.91 ± 0.08				
September	0.72 ± 0.10	0.94 ± 0.13	0.82 ± 0.09	0.92 ± 0.12				
November	0.81 ± 0.10	0.91 ± 0.13	0.82 ± 0.08	1.06 ± 0.18				
<i>c) N<sub>2</sub>O<sub>DEA</sub> (μg N-N<sub>2</sub>O.g<sup>-1</sup>.h<sup>-1</sup>)</i>								
May	1.17 ± 0.04	1.39 ± 0.13	1.51 ± 0.22	1.39 ± 0.01	***	<b>0.022</b>	<b>&lt;0.001</b>	0.712
July	1.05 ± 0.07	1.15 ± 0.09	1.15 ± 0.01	1.30 ± 0.09				
September	1.19 ± 0.06	1.15 ± 0.06	1.21 ± 0.13	1.28 ± 0.10				
November	0.98 ± 0.04	1.03 ± 0.07	1.03 ± 0.06	1.19 ± 0.1				
<i>d) N<sub>2</sub>O<sub>DEA</sub> (μg N-N<sub>2</sub>O.g<sup>-1</sup>.h<sup>-1</sup>)</i>								
May	0.07 ± 0.04	0.04 ± 0.01	0.06 ± 0.01	0.17 ± 0.09	**	0.555	<b>&lt;0.001</b>	0.557
July	0.08 ± 0.04	0.19 ± 0.05	0.20 ± 0.06	0.09 ± 0.03				
September	0.08 ± 0.03	0.27 ± 0.11	0.25 ± 0.15	0.19 ± 0.04				
November	0.29 ± 0.04	0.29 ± 0.05	0.38 ± 0.09	0.37 ± 0.03				
<i>e) Denitrification product ratio (% N<sub>2</sub>O<sub>DEA</sub>/[N<sub>2</sub>O<sub>DEA</sub>+N<sub>2</sub>O<sub>DEA</sub>])</i>								
May	93.1 ± 2.2	97.7 ± 2.2	96.8 ± 2.1	95.7 ± 1.9	***	0.129	<b>&lt;0.001</b>	0.197
July	92.0 ± 3.6	85.8 ± 3.8	85.5 ± 3.6	93.1 ± 2.4				
September	93.3 ± 2.2	81.6 ± 10	80.1 ± 5.8	86.7 ± 3.6				
November	77.2 ± 2.6	77.8 ± 4.2	73.8 ± 8.9	75.7 ± 2.1				

Abbreviations: ANOVA, analysis of variance; C, control treatment; T, elevated temperature treatment; TD, temperature and drought treatment; TDCO<sub>2</sub>, temperature drought and elevated CO<sub>2</sub> treatment; NO<sub>3</sub><sup>-</sup>, nitrate pools measured by NEA; NEA, nitrifying enzymatic activities; N<sub>2</sub>O<sub>DEA</sub>, potential N<sub>2</sub>O fluxes measured by denitrification; N<sub>2</sub>O<sub>DEA</sub>, potential N<sub>2</sub> fluxes measured by denitrification. \*, \*\*, \*\*\* Indicates significant differences at  $P < 0.05$ , 0.01, or 0.001, respectively and *italic* indicates marginal differences  $P < 0.1$

**Table 3: Effects of climate change treatments on the (a) AOB, (b) nirK and (c) nosZ gene abundances (means and standard errors are shown; n = 5). Results from repeated measures ANOVA testing the effects of climate change treatments, measurement dates and their interaction are presented (significant P values are shown in bold).**

	Climate treatments				Repeated measures ANOVA			
	C	T	TD	TDCO <sub>2</sub>	p-model	Treatments	Dates	Treatments x Dates
<i>a) AOB number, abundance of ammonia-oxidizing bacteria (10<sup>6</sup> copy)</i>								
May	3.97 ± 0.15	5.58 ± 0.67	4.34 ± 0.80	4.13 ± 0.15	***	0.083	<0.001	<b>0.021</b>
July	4.84 ± 0.70	4.95 ± 0.46	4.77 ± 0.69	5.32 ± 0.76				
September	5.14 ± 0.39	4.73 ± 0.50	4.40 ± 0.66	5.01 ± 0.32				
November	5.12 ± 0.43	6.40 ± 0.59	7.23 ± 0.54	8.36 ± 0.86				
<i>b) nirK number, abundance of Cu nitrite reductors (10<sup>7</sup> copy)</i>								
May	1.70 ± 0.23	1.93 ± 0.12	1.48 ± 0.14	1.64 ± 0.18	ns	0.371	0.051	0.740
July	2.10 ± 0.38	2.06 ± 0.35	2.31 ± 0.37	2.22 ± 0.33				
September	1.62 ± 0.25	2.20 ± 0.33	1.82 ± 0.31	1.94 ± 0.19				
November	1.36 ± 0.23	1.76 ± 0.26	1.98 ± 0.24	2.03 ± 0.84				
<i>c) nosZ number, abundance of nitrous oxide reductors (10<sup>6</sup> copy)</i>								
May	2.33 ± 0.35	2.33 ± 0.09	1.93 ± 0.47	2.60 ± 0.51	*	<b>0.028</b>	<b>0.009</b>	0.576
July	2.33 ± 0.09	2.69 ± 0.38	2.78 ± 0.48	2.33 ± 0.63				
September	1.93 ± 0.47	2.73 ± 0.43	2.76 ± 0.47	2.79 ± 0.71				
November	2.60 ± 0.51	3.52 ± 0.54	3.79 ± 0.46	3.40 ± 0.40				

Abbreviations: ANOVA, analysis of variance; C, control treatment; T, elevated temperature treatment; TD, temperature and drought treatment; TDCO<sub>2</sub>, temperature drought and elevated CO<sub>2</sub> treatment. \*, \*\*, \*\*\* Indicates significant differences at P < 0.05, 0.01, or 0.001, respectively and italic indicates marginal differences (P < 0.1)

#### 4.5 Relationship between microbial activities, microbial population abundances and abiotic factors

Across treatments, field N<sub>2</sub>O fluxes showed a positive correlation with N<sub>2</sub>O<sub>DEA</sub> and denitrification product ratio (Table 4) but a negative correlation with N<sub>2</sub>DEA during the study period. No significant relationships were observed between N<sub>2</sub>O fluxes and gene abundances across treatments. However, correlations between microbial activities, microbial populations and N<sub>2</sub>O fluxes varied depending on climate treatment, and variation in gene abundance played a relatively more important role for N<sub>2</sub>O fluxes under warmed conditions (Table 4). In the control treatment, N<sub>2</sub>O fluxes were positively correlated with the denitrification product ratio and N<sub>2</sub>O<sub>DEA</sub>. In contrast, N<sub>2</sub>O fluxes in the C treatment showed a significant negative correlation with both NEA and N<sub>2</sub>DEA. In the T treatment, N<sub>2</sub>O fluxes showed a significant negative correlation with both NEA and the nosZ / nirK ratio (Table 4). In the TD treatment, N<sub>2</sub>O fluxes were positively correlated with the denitrification product ratio but negatively

correlated with *nosZ* abundance. Finally in the TDCO<sub>2</sub> treatment, N<sub>2</sub>O fluxes showed a positive correlation with the denitrification product ratio but a negative correlation with N<sub>2</sub>DEA and the *nosZ* / *nirK* ratio (Table 4).

**Table 4: Correlation coefficients (Spearman) for the relationship between field N<sub>2</sub>O fluxes and microbial activities and gene abundances across experimental climate treatments (n = 80) and for each climate treatment (n = 20). Significant P values (P < 0.05) are shown in bold and marginal P values (0.1 > P > 0.05) are in italic.**

Field N <sub>2</sub> O fluxes	Microbial activities				Gene abundances			
	NEA	N <sub>2</sub> O <sub>DEA</sub>	N <sub>2</sub> DEA	Denitrification product ratio	AOB	<i>nirK</i>	<i>nosZ</i>	<i>nosZ/nirK</i>
All treatments	0.016	<b>0.335</b>	<b>-0.291</b>	<b>0.411</b>	-0.179	0.117	-0.166	-0.216
C	<b>-0.462</b>	<b>0.477</b>	<b>-0.734</b>	<b>0.768</b>	-0.205	0.281	0.017	-0.211
T	<b>-0.428</b>	0.221	<i>-0.321</i>	<b>0.398</b>	0.006	<i>0.359</i>	-0.177	<b>-0.426</b>
TD	0.206	0.275	<i>-0.356</i>	<b>0.475</b>	-0.314	-0.252	<b>-0.447</b>	<b>-0.327</b>
TDCO <sub>2</sub>	-0.101	0.305	<b>-0.393</b>	<b>0.599</b>	<i>-0.346</i>	0.120	-0.264	<b>-0.394</b>

## 5- Discussion

Microbial processes are critical for dynamics and function of grassland ecosystems (Vitousek *et al.* 1997). Throughout our study, we found that microbial activities responded more strongly to warming and combined climate change (simultaneous application of warming, summer drought and elevated CO<sub>2</sub>) than to summer drought or elevated CO<sub>2</sub> under warmed conditions. In addition, similar patterns of response to warming and combined warming, summer drought and elevated CO<sub>2</sub> were observed for field N<sub>2</sub>O fluxes. Flux data from the present study confirms the importance of warming as a key driver of climate-induced changes for N<sub>2</sub>O-N losses in grassland ecosystems (Cantarel *et al.* 2010).

Irrespective of measurement date, both NEA and the nitrate pool (produced by nitrification) showed an increase in response to elevated temperature, consistent with results from other studies in well-aerated soils (Davidson *et al.* 2005, Barnard & Leadley 2005). In theory, DEA products (N<sub>2</sub>O<sub>DEA</sub> and N<sub>2</sub>DEA) should also increase under warming, as demonstrated by many soil incubation experiments (Maag & Vinther 1996, Castaldi 2000, Dobbie & Smith 2001). However, in the present study, DEA products did not respond to warming. Such a lack of response did not result from confounding effects of soil moisture since similar soil moisture conditions were observed in the C and T treatment during the



measurement campaigns. In a recent review, Barnard & Leadley (2005) found that DEA was generally less responsive to temperature in field experiments compared to laboratory studies, a phenomenon attributed to acclimation of DEA to ambient environmental conditions over time (French *et al.* 2009). These results highlight the importance of microbial field experiments to understand the relationship between temperature and microbial processes in natural ecosystems.

Compared with warming alone, combined summer drought and warming had no significant effect on enzymatic activities recorded during our study. This finding is somewhat unexpected since decreases in soil moisture are often associated with a decrease in DEA and an increase in NEA products (Linn & Doran 1984; Bollmann & Conrad 1998; Batemann & Baggs 2005; Ma *et al.* 2008). Part of the explanation for our results may lie with the weak variations in water status across T and TD treatments during the measurement campaigns. Only the September campaign showed a significant difference in soil water status with a 15 % reduction of WFPS. Consequently, the impact of experimental drought on soil processes may have been attenuated by soil moisture conditions during measurement campaigns. The lack of response to drought observed here for enzymatic activities mirrors patterns of N<sub>2</sub>O fluxes recorded at regular intervals over two years for the same site (Cantarel *et al.* 2010), suggesting that N<sub>2</sub>O-related microbial processes may be relatively insensitive to small changes in precipitation levels associated with out drought manipulation treatment. Measurement of enzymatic activities following drying/rewetting events is required to provide further insight into N<sub>2</sub>O emissions under future, drier conditions at this grassland site.

In contrast to summer drought, elevated CO<sub>2</sub> may have a positive effect on soil water status due to reduced plant stomatal aperture and transpiration rates (Schulze 1986). Such increases in soil water have consequences for the soil O<sub>2</sub> partial pressure, with direct impacts on N<sub>2</sub>O release by denitrification (Bollmann & Conrad 1998). In our experiment, the TD treatment had significantly lower soil moisture conditions in July and September compared with the TDCO<sub>2</sub> treatment (45 versus 53% and 31 % versus 49% for July and September respectively). However, we found no impact of elevated CO<sub>2</sub> on enzymatic activities and N<sub>2</sub>O fluxes, under warmed and droughted conditions. Although the absence of response of NEA and N<sub>2</sub>O fluxes to elevated CO<sub>2</sub> is consistent with other field experiments (Niklaus *et al.* 2001; Barnard *et al.* 2004, 2006) The lack of response of DEA products to elevated CO<sub>2</sub> is surprising, but consistent with the lack of response of N<sub>2</sub>O fluxes to elevated CO<sub>2</sub>.

Confounding effects of plant-soil competition for N could mask CO<sub>2</sub>-induced changes in microbial activities (De Graaf *et al.* 2006), but this remains to be tested.

Changes in microbial population abundance and community structure can modify microbial activities and have important consequences for ecosystem functioning and biogeochemical cycling (Schimel & Mikan 2005; Smits *et al.* 2010). In our study, microbial gene abundances showed less response to climate treatments than microbial activities, suggesting an increase in the efficiency of microbial processes for a given population size. Nevertheless, gene abundances showed significant climate treatment effects, driven primarily by responses to the TDCO<sub>2</sub> treatment. In general, we found that climate change treatments had a greater effect on the population size of denitrifiers compared with nitrifying microbes. Since the *nosZ* gene encoding the catalytic subunit of the nitrous oxide reductase determines the reduction of N forms to N<sub>2</sub>O or N<sub>2</sub> under denitrifying conditions, its presence and expression is crucial for N<sub>2</sub>O emissions from soil (Philippot *et al.* 2010). Furthermore, the population size of *nosZ* denitrifiers showed a stronger response than *nirK* denitrifiers to climate change drivers, *nosZ* abundances increasing under warming and combined climate change. These results imply that climate change could have significant effects on microbial community structure in soils where *nosZ* microbes are present, and that microbial response is not simply restricted to *nirK* populations as previously suggested (Hallin *et al.* 2009; Szukics *et al.* 2010).

Unlike *nosZ*, responses of AOB nitrifier population size to climate treatment varied with measurement date. Warming had a positive impact on AOB abundance in May whereas combined warming, drought and elevated CO<sub>2</sub> had a positive impact on AOB abundance in November. Given the relatively limited changes in gene abundance observed, and their transient nature, it is likely that the change in abundance of AOB that we observed was probably a result of indirect effects, most likely mediated by the plant community (Horz *et al.* 2004). AOB are known to be inferior competitors for some resources (Belsler 1979), and substantial changes in AOB community size could be indirectly linked with modifications in competition for nutrient resources between AOB, heterotrophic microbes and plants.

Across climate treatments, field N<sub>2</sub>O fluxes showed a stronger correlation with enzymatic activities than community abundance. N<sub>2</sub>O fluxes variations were better explained by the denitrification product ratio. Changes in DEA products over time (i.e. decrease of

N<sub>2</sub>O<sub>DEA</sub> and increase of N<sub>2</sub>DEA) were correlated with a decrease in field N<sub>2</sub>O fluxes over time, and almost certainly reflected concurrent changes in soil water content over measurement campaigns (Szikics *et al.* 2010). The denitrification product ratio was also most strongly correlated with field N<sub>2</sub>O fluxes under cool, wet conditions (control site). However, the relative importance of microbial activities and microbial population size was modified under warmed conditions, with stronger correlations being observed between field N<sub>2</sub>O fluxes and gene abundances in the T, TD and TDCO<sub>2</sub> treatments. Links between N<sub>2</sub>O fluxes and microbial abundances are known to be elusive, and may depend on soil properties or ecosystem type (Ma *et al.* 2008, Rich & Myrold 2004). Further work is needed to better understand the importance of microbial population size for N<sub>2</sub>O emissions under climate change.

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## ***Discussion Générale***

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Cette thèse s'inscrit dans une démarche générale de recherche sur l'évolution de la structure et du fonctionnement d'un écosystème prairial sous l'impact d'un scénario de changement climatique. Nous avons choisi d'aborder cette question en explorant l'évolution du couvert végétal, d'une part, et des flux de gaz à effet de serre (gaz carbonique et oxyde nitreux) d'autre part, en dissociant les différents facteurs du changement climatique (température, précipitations) et atmosphérique (teneur en CO<sub>2</sub>). Nos objectifs étaient (1) de déterminer l'importance de ces facteurs sur le fonctionnement et la structure d'un écosystème prairial et (2) d'identifier les processus et les mécanismes régissant ces modifications.

A partir d'un schéma conceptuel des impacts du changement climatique sur l'écosystème prairial construit sur l'état des connaissances au démarrage de cette thèse (voir Introduction Figure 18), nous avons identifié les lacunes majeures des connaissances et les points de blocages à notre compréhension de la dynamique de ces systèmes. Notre démarche scientifique s'est construite en considérant deux volets expérimentaux. Le premier volet est essentiellement basé sur l'analyse des réponses au changement climatique du couvert végétal d'une prairie permanente gérée extensivement<sup>1</sup>. Les variables et indicateurs suivis ont été la production aérienne, la structure du couvert, la diversité floristique, les échanges gazeux en CO<sub>2</sub> et la dynamique des sucres et de l'azote non structuraux chez une espèce modèle afin de rendre compte des variables d'état et des flux associés au fonctionnement de l'écosystème prairial étudié. Le second volet s'est attaché à caractériser les flux d'oxyde nitreux (N<sub>2</sub>O) sous changement climatique en termes de dynamique saisonnière et interannuelle et d'écologie microbienne. Le N<sub>2</sub>O est un gaz à effet de serre majeur de par son pouvoir de réchauffement, de plus il est connu pour être sensible à certains facteurs du changement climatique (*i.e.* température et précipitations) et aucune étude ne l'a encore analysé dans un dispositif multifactoriel de changement climatique. La question globale de la thèse a été abordée en considérant quatre problématiques principales :

i) déterminer comment évoluent au cours du temps, les effets du changement climatique sur la production de biomasse d'un couvert prairial et si ces modifications s'expliquent par des variations de la structure de la communauté (*i.e.* diversité spécifique, diversité fonctionnelle, traits fonctionnels);

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<sup>1</sup> Deux fauches par an, pas de fertilisation.

ii) mettre en évidence l'importance des processus physiologiques (*i.e.* photosynthèse, balance carbone/azote au sein de la plante) dans les réponses observées du couvert au changement climatique en décomposant les effets des facteurs (*i.e.* réchauffement *vs* augmentation du CO<sub>2</sub>);

iii) caractériser les flux de N<sub>2</sub>O au champ et leurs dynamiques saisonnières et interannuelles sous changement climatique;

iv) déterminer l'impact du changement climatique sur l'écologie microbienne associée aux flux de N<sub>2</sub>O et identifier les principaux processus microbiens à l'origine des flux mesurés à l'échelle du couvert en prairie.

Avant d'aborder la synthèse de ces résultats et de discuter leurs implications, nous rappellerons brièvement les principales conclusions :

i) Cinq années d'application d'un scénario climatique (*i.e.* + 3,5°C de température de l'air, -20 % de réduction des précipitations estivales et + 200 ppm en CO<sub>2</sub> atmosphérique) correspondant aux prévisions à l'horizon 2080 en Auvergne ont significativement réduit la production de biomasse annuelle d'une prairie de moyenne montagne extensivement gérée. L'analyse statistique montre que le réchauffement de 3,5°C de la température est à l'origine de cet effet. Cette baisse de production s'est accompagnée d'une modification de l'abondance relative des groupes fonctionnels (réduction des graminées et augmentation des légumineuses). Cependant, la diversité spécifique (*i.e.* richesse spécifique, indice de Shannon-Weaver) n'a montré aucune variation significative au cours de ces cinq années d'expérimentation.

ii) Les changements observés dans la production de biomasse du couvert prairial sous changement climatique s'accompagnent également de modifications de la structure du couvert : diminution de la hauteur et de l'indice foliaire (LAI) du couvert. De plus, les modifications de photosynthèse aux échelles de la feuille et du couvert indiquent une réduction du bilan de carbone dans les traitements réchauffés, y compris en cas d'enrichissement en CO<sub>2</sub>. En effet, les effets à long terme de cet enrichissement sont bien moindres que les effets à court terme, ce qui indique une acclimatation négative. Ces résultats soulignent que l'ensemble des composantes du bilan de carbone est touché par le

réchauffement, principalement *via* la baisse de l'interception du rayonnement et *via* l'acclimatation négative de la photosynthèse. L'étude des pools solubles de carbone et d'azote dans les feuilles d'une graminée dominante (*F. arundinacea*) souligne une explication possible pour ces changements : l'augmentation du ratio C:N soluble peut en effet à la fois réduire : i) l'allocation de la croissance au compartiment aérien (équilibre fonctionnel, Hilbert & Reynolds 1991; Maire *et al.* 2009) et ainsi l'indice foliaire ; ii) l'expression des protéines photosynthétiques et en particulier celle de la Rubisco. Même si nous ne disposons pas encore des résultats sur ce dernier point, cette hypothèse permet d'expliquer les principales observations.

iii) Les flux de N<sub>2</sub>O sont hautement variables à l'échelle de la saison comme à l'échelle de l'année. L'augmentation des températures de l'air contribue à accroître significativement les flux annuels de N<sub>2</sub>O. Ni la sécheresse estivale, ni l'augmentation des concentrations atmosphériques en CO<sub>2</sub> n'ont d'effets sur ces flux. Cependant, les émissions de N<sub>2</sub>O les plus fortes ont été enregistrées pour le traitement combinant toutes les variables du changement climatique, montrant une interaction positive entre le réchauffement et l'enrichissement en CO<sub>2</sub>.

iv) Le réchauffement favorise les activités enzymatiques de nitrification et de dénitrification des bactéries, ainsi que les abondances de gènes (*i.e.* AOB et *nosZ*) bactériens liés à la production de N<sub>2</sub>O. Nous montrons que le ratio de dénitrification<sup>2</sup> est le meilleur indicateur des flux de N<sub>2</sub>O mesurés à l'échelle du couvert. Lors d'une dénitrification totale (*i.e.* production de N<sub>2</sub>), nous trouvons peu de flux de N<sub>2</sub>O *in situ*. Ce résultat met en évidence que même si il n'y a pas de flux de N<sub>2</sub>O mesurable *in situ*, les pertes azotés du système sont toujours présentes (*i.e.* N<sub>2</sub>). Nos résultats soulignent que l'importance de la taille des communautés (*i.e.* pool de gènes liés à la production de N<sub>2</sub>O) microbiennes va aussi jouer sur les flux de N<sub>2</sub>O mesurés *in situ* pour les traitements climatiques réchauffés.

Ce travail de thèse s'attache à coupler les processus et les mécanismes à l'échelle sol-plante-atmosphère. C'est pour cela que nous avons conçu un dispositif expérimental additif comprenant quatre traitements climatiques (témoin (C), température (T), température et

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<sup>2</sup> Ratio entre les flux de N<sub>2</sub>O et de N<sub>2</sub> produit par dénitrification (%)



sécheresse (TD) et température, sécheresse et CO<sub>2</sub> (TDCO<sub>2</sub>) ; voir Introduction Encadré 1). L'intérêt de ce dispositif expérimental est de pouvoir tester les principaux facteurs du changement climatique (*i.e.* effet réchauffement, effet sécheresse ou encore effet augmentation du CO<sub>2</sub> atmosphérique) tout en conservant une vision globale et intégrative du changement climatique, correspondant à l'effet combiné de tous les facteurs. Cette double approche est en effet indispensable pour pouvoir conclure sur l'impact d'un scénario de changement climatique futur à l'échelle de l'écosystème. Aucune démarche expérimentale ne permet de reproduire avec fidélité les changements climatiques qui sont supposés se dérouler dans le futur. C'est une des limites de notre approche. De plus les incertitudes sur les scénarios climatiques sont encore fortes. Un autre point à considérer est la concentration atmosphérique en CO<sub>2</sub> et la température moyenne du globe qui augmentent lentement et progressivement ; chose impossible à reproduire en situation expérimentale et encore plus dans une approche analytique. Ainsi le matériel végétal présent à l'horizon 2080 sera vraisemblablement différent de celui d'aujourd'hui, car il aura pu s'adapter aux modifications progressives de son environnement. Un point souvent avancé, est que cette adaptation sera supportée par une dérive génétique des populations naturelles, ou influencée par des phénomènes exogènes comme l'invasion ou la migration. En tout état de cause et compte tenu du peu d'expérimentations multifactorielles *in situ* étudiant l'impact du changement climatique sur les écosystèmes et de l'importance de prédire les modifications des écosystèmes en réponse au changement climatique, notre démarche expérimentale peut être qualifiée d'originale et de novatrice.

L'objet de cette discussion est de préciser les apports de ce travail à la compréhension des impacts du changement climatique sur l'écosystème prairial en mettant en relation les résultats apportés par les différents chapitres expérimentaux. La démarche adoptée durant cette thèse, c'est à dire la volonté d'étudier les processus et mécanismes de l'écosystème prairial dans leur globalité, nous a permis d'apporter des propositions pour conceptualiser le fonctionnement et la réponse de l'écosystème prairial. Dans un premier temps, nous nous concentrerons sur les effets du changement climatique sur le couvert prairial, afin de discuter les éléments apportés par l'analyse de l'évolution à long-terme du fonctionnement et de la structure du couvert et l'importance des processus physiologiques dans les réponses du couvert au changement climatique (chapitre 1 et 2). Nous considérerons en suite les apports

de nos résultats sur les flux de N<sub>2</sub>O en prairie en relation avec l'écosystème microbien associé (chapitre 3 et 4).

## 1- Fonctionnement et structure d'un couvert végétal sous changement climatique en prairie

Le climat induit des changements dans le fonctionnement de l'écosystème (*i.e.* production de biomasse) *via* des effets directs sur la végétation, en modifiant les processus physiologiques, (*i.e.* photosynthèse) et / ou *via* des effets indirects sur la structure (Lavorel & Garnier, 2002) de la communauté végétale, notamment en modifiant la richesse spécifique, l'abondance relative des groupes fonctionnels ou les traits fonctionnels des plantes. Malgré une augmentation des expérimentations *in situ* de changement climatique au cours de ces 10 dernières années (cf. chapitre 1), les réponses de l'écosystème au changement climatique à long terme (> 3 ans) restent encore mal connues (Dukes *et al.* 2005). Cela est d'autant plus vrai lorsque l'on s'intéresse aux effets progressifs<sup>3</sup> du changement climatique sur la production de l'écosystème. Dans notre travail, la combinaison de toutes les variables climatiques (température, sécheresse estivale et concentrations en CO<sub>2</sub>)<sup>4</sup> a montré des effets positifs sur la production de biomasse du couvert la première année de l'expérimentation. Ces effets sont strictement liés à l'influence du réchauffement sur la production (Figure 1). Cependant, ces effets sont devenus négatifs après trois années d'expérimentations (cf chapitre 1). Ainsi en 2009, la réduction de biomasse annuelle comparée aux conditions climatiques actuelles était de -30% sous réchauffement et de -15 % sous changement climatique (Figure 3 du chapitre 1). Ces résultats confirment l'importance du réchauffement comme le facteur clés des modifications induites par le changement climatique sur la production annuelle de biomasse (Rustad *et al.* 2001 ; Bloor *et al.* 2010). Ils posent en outre la question de l'interrelation entre la hausse de croissance du couvert prairial observé en début d'étude et sa capacité à absorber l'azote du sol. Ainsi, la réduction de la production dès la seconde année d'expérimentation peut être imputable à une impossibilité pour les plantes de prélever l'azote

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<sup>3</sup> De nombreuses études ont mis en évidence des temps de réponses ou des effets progressifs des facteurs climatiques tel que les précipitations ou les teneurs en CO<sub>2</sub> sur les réponses de la production aérienne (Winkler & Herbst 2004 ; Kamman *et al.* 2005 ; Sherry *et al.* 2008)

<sup>4</sup> L'effet du changement climatique global, c'est à dire de tous les facteurs climatiques amenant à la réalisation du scénario climatique ACACIA A2, sera à partir de cet instant simplement appelé changement climatique.

du sol et en conséquence de reconstituer leurs réserves à une vitesse suffisamment rapide pour entretenir ce surplus de biomasse (Arft *et al.* 1999). Cette hypothèse est soutenue par des travaux récents (Fontaine *et al.* 2011) qui montre que lorsque l'assimilation des ressources nutritives du sol par les plantes est élevée, l'extraction microbienne de matière organique du sol (MOS) peut être intensifiée. Cela conduit à une destruction plus importante de la MOS et à une augmentation des ressources nutritives disponible pour les plantes et pour les microorganismes. Inversement, lorsque l'assimilation des ressources nutritives du sol par les plantes est faible, l'immobilisation microbienne de la ressource azotée augmente. Il en résulte une séquestration supérieure des ressources nutritives dans la MOS et une diminution des ressources disponibles pour les plantes. Les données sur le couvert végétal en 2009 nous confirment que le couvert obtenu est moins haut (cf. Tableau 2 chapitre 2) et moins dense (réduction du LAI ; cf. Tableau 2 chapitre 2) durant toute la saison de croissance en conditions réchauffées. La biomasse racinaire mesurée cette même année est affectée par le réchauffement. Cela se traduit par une diminution significative de la biomasse des racines sous réchauffement (Thèse R. Pilon, en cours). De plus, cette même année (*i.e.* 2009) une immobilisation accrue dans la matière organique agrégée a été mis en évidence dans les traitements réchauffés (Thèse R. Pilon, en cours). Nos résultats ont mis en lumière une augmentation du stress hydrique *via* une augmentation de l'évapotranspiration du couvert (cf. Annexe 2 chapitre 2) sous traitements réchauffés. Ce stress hydrique peut jouer sur la disponibilité en azote du sol *via* une diminution de la diffusion des ions nitrate et ammonium (Raynaud & Leadley 2004). Ces résultats tendent à confirmer l'hypothèse qu'en condition de réchauffement, les plantes ont un accès plus difficile aux réserves azotées du sol.

Les modifications de biomasse sous réchauffement et changements climatiques peuvent aussi s'expliquer par des effets directs des facteurs climatiques sur la physiologie du couvert (Figure 1). Durant une saison de croissance (*i.e.* de mai à septembre 2009 ; cf. chapitre 2), nous avons mesuré les échanges gazeux en CO<sub>2</sub> à l'échelle de la canopée végétale et à l'échelle de l'individu (*i.e.* *Festuca arundinacea*). Nous avons également suivi l'allocation des ressources carbonées au sein de la plante (*i.e.* relation source / puits entre limbes et gaines). Lors de cette étude, le réchauffement a eu un effet négatif sur la photosynthèse à l'échelle du couvert. Ces résultats sont à mettre en relation avec la réduction de la surface foliaire disponible pour l'interception lumineuse (cf. Tableau 2 chapitre 2).

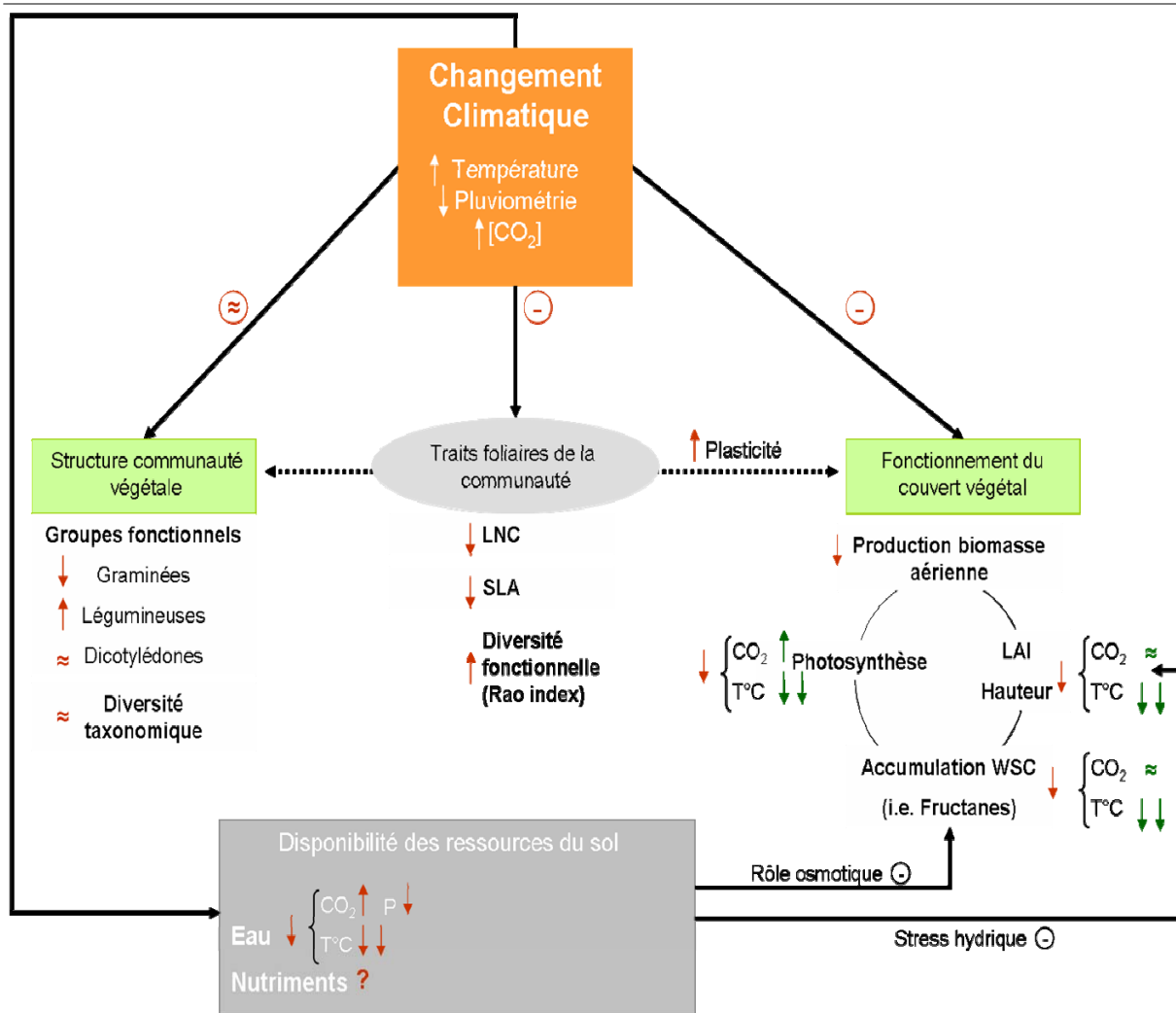


Figure 1 : Impact du changement climatique sur le fonctionnement d'un couvert prairial de moyenne montagne extensivement géré. Modèle conceptuel du premier volet de thèse à partir des résultats des chapitres 1 et 2.

Le réchauffement peut également avoir des effets indirects sur la photosynthèse du couvert car il augmente son évapotranspiration. A terme, cela accroît le stress hydrique sous réchauffement (Shah & Paulsen) et affecte la structure du couvert (LAI et hauteur). Ce stress hydrique peut induire l'hydrolyse des fructanes, mécanisme identifié pour maintenir la pression osmotique dans les cellules végétales (Wiemken *et al.* 1995). A *contrario* la formation de fructane a des effets protecteurs sur les membranes des cellules végétales lors de stress hydrique (Amiard *et al.* 2003) ; et donc sur la structure du couvert. Dans le cadre de notre étude, le rôle osmotique des fructanes est prépondérant. Nous avons trouvé une diminution significative de la concentration en fructane dans les limbes de *F. arundinacea* et une augmentation de la concentration en fructose (*i.e.* produit de l'hydrolyse des fructanes) sous réchauffement. Ces adaptations physiologiques (*i.e.* réponses de la photosynthèse, modifications des réponses carbonées) et structurelles (*i.e.* production de biomasse) peuvent

être résumées par l'hypothèse d'un accès plus difficile aux réserves hydriques et azotées du sol

Elles vont aussi modifier les capacités de compétition des plantes, et donc influencer sur la composition botanique de la communauté (Niu *et al.* 2008). On peut donc s'attendre à une modification des dominances spécifiques au sein de la végétation. Toutefois, après quatre ans d'expérience, ni la richesse spécifique, ni la diversité taxonomique de la communauté végétale n'ont évolué significativement sous changement climatique. Cependant, le changement climatique et le réchauffement entraînent des modifications dans les abondances relative des groupes fonctionnels (*i.e.* Graminées, Dicotylédones, Légumineuses) après trois ans, avec une diminution significative de la proportion des graminées (-14 % ; cf. Tableau 2 chapitre 1) et une augmentation de la proportion des légumineuses (+10 %). La diversité fonctionnelle est connue pour répondre plus rapidement que la diversité taxonomique (Suding *et al.* 2008). Mais cela n'avait encore jamais été montré sous changement climatique, même si des changements rapides d'abondance de ces groupes ont été observés sous enrichissement en CO<sub>2</sub> (Teyssonneyre *et al.* 2002). Notre étude révèle que les traits fonctionnels et la diversité fonctionnelle (caractérisé par l'indice de Rao, cf. Chapitre 2) subissent des modifications plus rapidement que la diversité taxonomique sous changement climatique. Les traits foliaires agrégés à l'échelle de la communauté, que sont la surface spécifique foliaire (SLA) et la teneur en azote des limbes (LNC), sont négativement affectés par le changement climatique (Tableau 3 du chapitre 1). La diversité fonctionnelle augmente avec le changement climatique ce qui suggère une augmentation de la plasticité phénotypique des individus soumis au changement climatique, pouvant tamponner les modifications de la structure des communautés et les interactions entre espèces (Owensby *et al.* 1999). Nos données de traits agrégés, d'abondance relative des groupes fonctionnels répondent globalement plus vite au changement climatique que la diversité taxonomique. Ceci est en accord avec l'hypothèse des espèces dominantes (*i.e.* the mass ratio hypothesis, Grime 1998) qui suggère que les caractéristiques fonctionnelles (*i.e.* traits) des espèces dominantes de la communauté déterminent le fonctionnement et les propriétés de l'écosystème (Diaz & Cabido 2001 ; Lavorel & Garnier 2002).

Les effets de la sécheresse estivale et de l'augmentation des concentrations en CO<sub>2</sub> ont aussi été étudiés. L'impact de la sécheresse estivale est fortement lié aux conditions climatiques de l'année en cours et des années précédentes, les années humides tamponnant les

effets attendus d'une réduction de l'apport d'eau (Sherry *et al.* 2008). L'impact d'une augmentation des teneurs atmosphériques en CO<sub>2</sub> sur le couvert prairial n'est apparue qu'en 2008 avec une augmentation de la production mais cet effet n'est pas suffisant pour contrebalancer l'effet négatif du réchauffement dans le traitement de changement climatique combiné. Cette observation se retrouve aussi à l'échelle du couvert puisque l'on observe en début de saison un effet positif du CO<sub>2</sub> sur le taux de photosynthèse du couvert. A l'échelle d'une graminée dominante, *F. arundinacea* l'effet positif du CO<sub>2</sub> n'a pas été observé au printemps et une acclimatation négative forte de la photosynthèse a été observée. Pour des raisons de méthodologie, notre étude n'a pu porter que sur l'une des espèces dominantes avec une taille de limbe suffisante. Ceci constitue une limite à notre étude, car nous savons que les graminées sont des espèces montrant des variations interspécifiques importantes (Soussana *et al.* 2005, Niu *et al.* 2008). En ce sens, pour généraliser nos résultats, il serait intéressant d'améliorer le système de mesure des échanges gazeux en CO<sub>2</sub> à l'échelle foliaire, afin de pouvoir accroître la gamme des espèces considérées. Cela nous permettrait de mieux corrélérer les données obtenues à l'échelle du couvert avec celles obtenues à l'échelle de l'individu. Au final, il est intéressant de constater que l'augmentation de la concentration en CO<sub>2</sub> atmosphérique modifie la physiologie et la structure du peuplement de manière différente de l'augmentation des températures, les deux facteurs semblant antagonistes. Ces modifications de physiologie et de structure ont des effets non additifs puisque nous ne retrouvons pas une atténuation de l'effet négatif du réchauffement par l'élévation en CO<sub>2</sub> dans le traitement où tous les facteurs climatiques sont combinés.

En conclusion, le changement climatique affecte le fonctionnement de l'écosystème (*i.e.* la production) *via* l'effet direct du réchauffement sur la physiologie, la structuration du couvert et *via* l'effet indirect du réchauffement sur les ressources hydriques et nutritives du sol.

## **2- Les flux d'oxyde nitreux (N<sub>2</sub>O) en prairie sous changement climatique**

Compte-tenu du fort pouvoir de réchauffement global du N<sub>2</sub>O (310 fois celui du CO<sub>2</sub>), il est impératif de mieux mesurer les valeurs de flux de ce gaz dans les systèmes prairiaux. Cette problématique est cruciale pour évaluer le rôle des sols prairiaux comme puits de gaz à effet

de serre (GES) dans ces systèmes. A notre connaissance aucune étude n'a analysé les flux de N<sub>2</sub>O *in situ* sous les effets combinés des facteurs du changement climatique. De plus, la gestion extensive à laquelle est soumis notre dispositif expérimental (*i.e.* pas de fertilisation), nous a permis d'étudier les flux de N<sub>2</sub>O indépendamment de l'effet stimulant de l'apport d'intrants azotés. En ce sens, nous ne considérons que les conditions abiotiques du milieu (*i.e.* température du sol, teneur en eau du sol) et l'action conjointe de l'écosystème microbien *via* la ressource azotée disponible du sol.

Nos données de flux de N<sub>2</sub>O mesurés dans les traitements sans modifications climatiques, sont du même ordre que les flux de N<sub>2</sub>O de prairie non fertilisée et non pâturée (Flécharde *et al.* 2007, Kammann *et al.* 2008). De 2007 à 2008, nous avons mesuré quelque soit le traitement climatique une forte variation intra-annuelle des flux de N<sub>2</sub>O. Les flux les plus importants sont mesurés en été et en automne (cf. Figure 2 chapitre 3). Ces résultats peuvent surprendre au vue des conditions abiotiques existantes à ces périodes : combinaison de températures élevées et de teneurs en eau du sol faibles (cf. chapitre 3). Nous avons trouvé des réponses similaires sous l'impact d'un réchauffement en 2009. En effet, les flux de N<sub>2</sub>O les plus élevés coïncident avec les températures les plus élevées (*i.e.* 17.3°C) et les teneurs en eau du sol les plus faibles (*i.e.* 31.9 % de WFPS<sup>5</sup> ; Figure et Tableau 1 du chapitre 4). Ces résultats suggèrent que la température du sol a une influence relativement importante sur des flux N<sub>2</sub>O en condition climatique ambiante. La température du sol pourrait jouer sur la stimulation des processus microbiens liés à la production N<sub>2</sub>O, comme la nitrification (*i.e.* favorisée en condition aérobie). Cependant en 2009, nous n'avons pas trouvé de changement de la nitrification au cours des différentes dates de prélèvement (*i.e.* différentes conditions de température du sol, cf. Tableau 2 chapitre 4). Cet effet de la température du sol pourrait être lié à une diffusion du N<sub>2</sub>O au travers du sol facilitée par les températures élevées (Hernandez-Ramirez *et al.* 2007). A l'échelle annuelle, les flux de N<sub>2</sub>O sont corrélés positivement avec la température du sol et la pluviométrie. L'importance des flux et de leurs variations sont alors fortement dépendantes des conditions climatiques moyennes, ainsi de fortes précipitations et des températures élevées au cours d'une année favoriseront les flux annuels de N<sub>2</sub>O. Pour être plus complet dans l'étude des dynamiques des flux de N<sub>2</sub>O, Flécharde *et al.* (2005) suggèrent de prendre en compte les variations annuelles, saisonnières et même journalières des flux de

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<sup>5</sup> Water Filled Pore Space

N<sub>2</sub>O. Pour se faire, il est nécessaire de développer de nouvelles expérimentations, comme les chambres de mesures du N<sub>2</sub>O automatisées qui permettent d'intégrer les flux de N<sub>2</sub>O d'une parcelle sur un pas de temps plus long que les chambres statiques.

En plus des variations annuelles et saisonnières, nous nous sommes attachés à étudier l'impact du changement climatique sur les flux de N<sub>2</sub>O *in situ*. Contrairement à nos attentes, ni la sécheresse estivale (combinaison de la température et la sécheresse), ni la combinaison de l'ensemble des facteurs climatiques (Figure 2) n'ont eu d'effets significatifs sur les flux annuels de N<sub>2</sub>O. Cela s'est vérifié en 2007, en 2008 et en 2009. Aucun effet sur les activités microbiennes n'a non plus été identifié en 2009. Les années 2007 et 2008 ont été très humides comparées aux années précédentes (*i.e.* + 46.3 % et + 28.9 % pour 2007 et 2008 respectivement comparé avec la moyenne des précipitations sur les trente dernières années). Par conséquent, l'impact du traitement de sécheresse estivale sur les processus du sol peut avoir été limité par ces conditions climatiques. Nous faisons l'hypothèse qu'avec des effets de sécheresse plus prononcés, des modifications des flux de N<sub>2</sub>O pourraient être observées. Pour la tester, nous suggérons de concevoir une expérience basée sur une manipulation du facteur sécheresse seul, avec différents taux d'apport d'eau. Cette expérience pourrait nous aider à mieux comprendre les effets tampons de la variabilité climatique *in situ*. De plus, il est bien connu que la forte variabilité spatiale et temporelle des flux N<sub>2</sub>O peut masquer les effets des traitements climatiques (Flechard *et al.* 2007). Ce type d'observation plaide en faveur du développement des expérimentations en chambres automatisées.

Au cours des deux années d'étude des flux de N<sub>2</sub>O, les flux les plus élevés ont été trouvés sous changement climatique. Cela suggère des pertes azotées supérieures dans des environnements enrichis en CO<sub>2</sub> et sous réchauffement. Ces émissions de N<sub>2</sub>O sont comparables aux pics d'émissions de certaines prairies fertilisées (*i.e.* 40 kg N ha<sup>-1</sup>y<sup>-1</sup>, voir Kammann *et al.* 1998). Nos résultats suggèrent que les réponses des flux de N<sub>2</sub>O sous changement climatique sont des réponses directes à l'effet du réchauffement et ponctuelles à l'effet du CO<sub>2</sub>. Ces pics ponctuels de N<sub>2</sub>O sous CO<sub>2</sub> élevé pourraient être à rattacher à l'augmentation des teneurs en eau du sol sous CO<sub>2</sub> élevé qui créent des conditions favorables pour la dénitrification. Les résultats acquis en 2009 sur les activités enzymatiques confirment cette hypothèse. D'un part, nous mesurons une augmentation des teneurs en eau du sol sous CO<sub>2</sub> élevé (+ 12 % entre Juillet et Septembre, cf. Tableau 1 chapitre 4) et d'autre part, les flux de N<sub>2</sub>O issus de la dénitrification sont favorisés sous changement climatique (cf. Tableau 2



chapitre 3). Ces pertes d'azote accrues sont à mettre en relation avec le fonctionnement du couvert végétal sous changement climatique. Elles pourraient être à l'origine d'une diminution de la disponibilité des ressources azotées du sol, en accord avec notre hypothèse d'un accès plus difficile aux réserves pour la végétation et être la cause de la diminution de production de biomasse aérienne de notre écosystème.

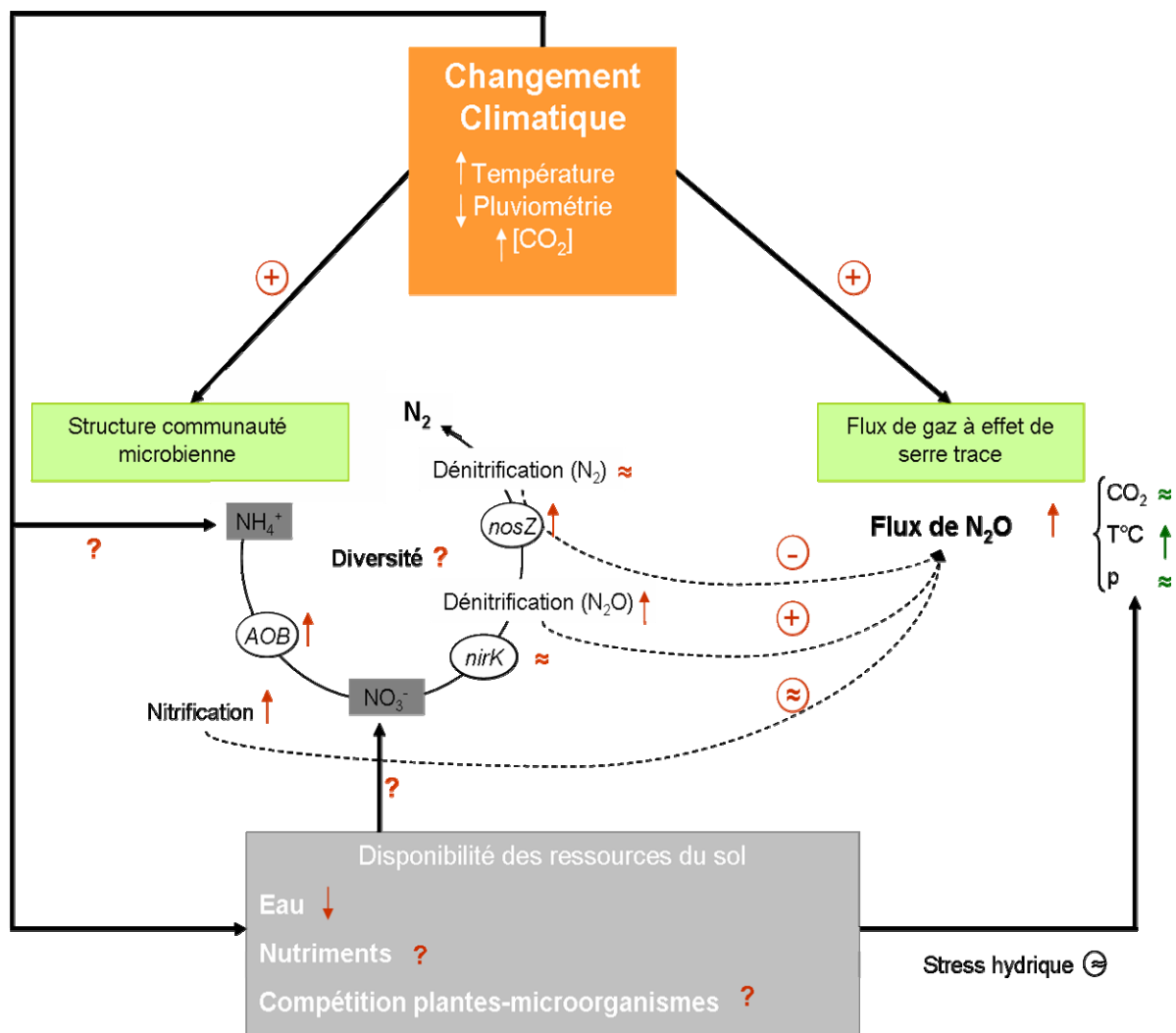


Figure 2 : Impact du changement climatique combiné sur les flux de  $N_2O$  et l'écosystème microbien associé. Modèle conceptuel du second volet de thèse à partir des résultats obtenus dans les chapitres 3 et 4

Le réchauffement a, en tendance, un impact positif sur les flux annuel de  $N_2O$  (2007 :  $P < 0.05$  ; 2008 :  $P < 0.1$ , cf. figure 3 du chapitre 3). Ces résultats se retrouvent en 2009. Nous avons sélectionné 3 dates de mesures en conditions favorables<sup>6</sup> pour les émissions de  $N_2O$ .

<sup>6</sup> Les conditions favorables pour les émissions de  $N_2O$  ont été déterminées à partir des observations des flux saisonniers (chapitre 3), les conditions favorables sont caractérisées par de fortes températures ambiantes.

Ces trois dates montrent l'effet positif du réchauffement sur les flux de N<sub>2</sub>O (cf. figure 1 du chapitre 4). Notre démarche nous a conduit à caractériser et comprendre l'évolution des mécanismes menant à la production de N<sub>2</sub>O *in situ* sous changement climatique. Nous montrons que la structure de la communauté microbienne était affectée par le changement climatique (Figure 2). Ainsi, les activités enzymatiques de nitrification et de dénitrification des microorganismes augmentent sous changement climatique (cf. Tableau 2 chapitre 4). La caractérisation des pools bactériens (cf. Tableau 3 chapitre 4), *via* l'analyse des abondances de gène bactérien, fait apparaître une augmentation des pools associés à la nitrification (*i.e.* AOB<sup>7</sup>) et à la formation de N<sub>2</sub> par dénitrification (*i.e.* nosZ<sup>8</sup>). En outre, il est surprenant de noter une absence de réponse du pool bactérien possédant le gène *nirK*<sup>9</sup> (*i.e.* enzymes produisant le N<sub>2</sub>O par dénitrification) de même qu'une augmentation de l'activité enzymatique dénitrifiante qui produit du N<sub>2</sub>O sous changement climatique. Nous avons envisagé une modification du pool microbien en réponse à une activité enzymatique plus importante. Cependant, ces résultats pourraient être liés à un changement dans la diversité des espèces possédant le gène *nirK* sous changement climatique (*i.e.* espèces plus productives sous changement climatique). Des analyses de diversité sur le gène *nirK* sont toujours en cours, et pourraient nous apporter des éclaircissements sur les modifications des processus de dénitrification sous changement climatique. Les flux de N<sub>2</sub>O mesurés *in situ* sont fortement corrélés au ratio de dénitrification. La modification des produits de fin de la dénitrification (N<sub>2</sub>O ou N<sub>2</sub>) est corrélée avec une diminution des flux de N<sub>2</sub>O *in situ*. Cela signifie qu'une diminution du N<sub>2</sub>O produit par dénitrification et une augmentation du N<sub>2</sub> produit par dénitrification engendre la diminution des flux de N<sub>2</sub>O *in situ*. Ces modifications reflètent essentiellement des changements de teneurs en eau du sol et de températures. Les flux de N<sub>2</sub>O via dénitrification semblent favorisés sous températures élevées et teneurs en eau du sol faibles. Inversement, les flux de N<sub>2</sub> issus de la dénitrification sont favorisés en conditions humides et fraîches. Cependant l'importance relative des pools microbiens dans la production des flux de N<sub>2</sub>O *in situ* augmente avec le réchauffement. Les relations entre les flux de N<sub>2</sub>O, les activités enzymatiques et les abondances microbiennes dépendent donc des propriétés abiotiques du sol (Ma *et al.* 2008), mais également de la compétition plantes-microorganismes pour la ressource azotée du sol. De nouvelles études sont nécessaires pour caractériser les

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<sup>7</sup>Gène impliqué dans la formation de N<sub>2</sub>O dans la nitrification, Ammonium Oxidizing Bacteria

<sup>8</sup> Gène impliqué dans la formation de N<sub>2</sub>O dans la dénitrification, Oxyde nitreux reductase Z

<sup>9</sup> Gène impliqué dans la formation de N<sub>2</sub>O dans la dénitrification, Nitrite réductase K

interactions plantes-microorganismes afin de mieux cerner les échanges entre le continuum sol-plantes-atmosphère. Nous développerons quelques pistes possibles dans la partie sur les perspectives ouvertes par ce travail.

### 3- Perspectives

Dans cette thèse, nous nous sommes focalisé sur l'étude de la vulnérabilité de l'écosystème prairial à un scénario moyen de changement climatique. Il s'agissait en premier lieu d'identifier l'effet des principaux facteurs du changement et d'évaluer les impacts engendrés par la complémentarité des facteurs (interactions, rétroaction, effet synergique). Pour ce faire, la considération d'un scénario moyen était suffisante. Cependant, l'évolution du contexte climatique sur les trois dernières années (GIEC 2007) montre clairement que la vulnérabilité climatique d'une part et l'augmentation des événements extrêmes d'autre part, sont des éléments indispensables à intégrer dans les travaux futurs. En conséquences et compte tenu du recul procuré par notre travail, nous pensons qu'il est nécessaire d'intégrer ces événements extrêmes, dans le cadre d'un scénario moyen. Cela permettra d'identifier les mécanismes de résistance, puis d'adaptations possibles, en réponse à ces perturbations climatiques. Par ailleurs, au delà du changement climatique, le concept de changement global se développe et intègre des modifications inhérentes aux modes d'utilisation des terres. Intégrer ces concepts dans nos modèles d'étude impose une capacité à identifier les scénarios de changement de pratiques testables expérimentalement. Ainsi, les modalités d'utilisation des terres, facteur connu pour avoir un impact fort sur la dynamique prairiale (Sala *et al.* 2000) devraient à l'avenir être couplé au changement climatique. Dans ce cadre, l'étude de la vulnérabilité peut se concevoir en considérant des approches expérimentales différentes. Des dispositifs en conditions naturelles sans manipulation climatique, permettant d'étudier l'impact de la variabilité intra et interannuelle des facteurs climatiques. Cela pourra se faire sur des dispositifs d'observation longue durée (du type ORE<sup>10</sup>). Dans ce cadre, les changements d'utilisation des terres s'étudient conjointement à la variabilité climatique 'naturelle' en concevant des gradients de perturbations. Les résultats collectés fournissent des données sur l'évolution et la vulnérabilité des écosystèmes prairiaux aux événements climatiques naturels (*i.e.* canicule de 2003 et 2005) sous contraintes de gestion. Le deuxième type de dispositif

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<sup>10</sup> ORE, Observatoire de Recherche Expérimentale, [http://www.inra.fr/presse/inauguration\\_ore\\_clermont](http://www.inra.fr/presse/inauguration_ore_clermont)

envisageables sont des dispositifs en conditions semi- ou totalement contrôlées autorisant une manipulation plus fine de ces systèmes. En conditions semi-contrôlées, des dispositifs expérimentaux ont été développés pour coupler changement d'utilisation des terres, scénario climatique moyen (température et pluviométrie) et événements extrêmes *in situ*. Cette démarche a été entreprise en 2009 au sein de l'UREP<sup>11</sup> dans le cadre du projet ANR-VALIDATE<sup>12</sup>. Ce projet tend à analyser l'impact d'un scénario moyen de changement climatique (température et précipitations) et d'extrêmes estivaux sous deux modes de gestion différents (fauches fréquentes, fauches peu fréquentes). Cette expérimentation se fait à concentrations atmosphériques en CO<sub>2</sub> constantes, et fournit des informations sur les réponses de l'écosystème en termes de résistance et de résilience aux événements extrêmes (*i.e.* du type de la canicule de 2003). Une autre démarche méthodologique est de revenir à des expériences en conditions totalement contrôlées, permettant d'augmenter la gamme et la précision des régulations environnementales et des mesures. En confinant les écosystèmes dans des enceintes, on permet la simulation d'une large gamme de conditions environnementales (température, conditions hydriques, teneur en CO<sub>2</sub>, polluants...) et la mesure précise des principaux flux générés. En sortie, ces systèmes permettent d'établir des bilans précis et intégratifs (*i.e.* prise en compte du système aérien et souterrain). C'est dans cette optique qu'une expérimentation est actuellement en cours à l'Ecotron<sup>13</sup> de Montpellier. Les dispositifs expérimentaux, développés dans les structures du type de l'Ecotron, permettront d'aborder un grand nombre de questions nouvelles, telles les interactions entre les diversités à différents niveaux trophiques à l'impact des polluants sur les écosystèmes (sol et végétation) dans les climats du futur.

Cependant, nous sommes conscients que les dispositifs expérimentaux malgré toutes les améliorations méthodologiques pouvant être effectuées, ne pourront répondre à toutes les questions relatives au changement climatique. La notion de changement progressif à l'échelle du siècle ne peut être prise en compte par l'expérimentation seule. La complexité des systèmes comme les écosystèmes prairiaux nécessite de coupler les approches expérimentales et de modélisation. La combinaison des approches permettra la formalisation des flux de

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<sup>11</sup> UREP, Unité de recherche sur l'écosystème prairial ; <https://www1.clermont.inra.fr/urep/fgep/index.htm>

<sup>12</sup> ANR-VALIDATE, Vulnérabilité des prairies permanentes à un événement climatique extrême (canicule, sécheresse) en conditions semi-contrôlées ; <http://www1.clermont.inra.fr/validate/index.php>

<sup>13</sup> Ecotron, <http://www.ecotron.cnrs.fr/>

données et de connaissances. Pour produire des résultats acceptables et fiables, les modèles nécessitent d'être 'alimentés' avec des données fiables en entrée (*i.e.* paramétrage) et à des fins de validation. Dans cette thèse, nous avons caractérisé le fonctionnement de l'écosystème prairial sous changement climatique. Le jeu de données produit est fiable et robuste et peut être utilisé pour paramétrer des modèles simulant l'écosystème prairial, tel le modèle PASIM<sup>14</sup> développé au sein de l'UREP. Nous espérons que ce jeu de données pourra à l'avenir être intégré à PASIM pour améliorer des lois de réponse pilotant le fonctionnement de l'écosystème et sa réponse aux facteurs climatiques. Cette intégration devrait permettre d'accroître la fiabilité des prédictions ou d'élargir la gamme des références visant à la validation. Le projet européen NitroEurope a pour objectif d'améliorer la simulation des flux de N<sub>2</sub>O. Notre jeu de données est actuellement utilisé par PASIM pour son paramétrage sur cet aspect. Les résultats préliminaires sont engageants, puisque les sorties du modèle en terme de simulation des flux de N<sub>2</sub>O par PASIM ne montre pas de différences importantes avec les valeurs mesurées in situ. Cependant, une amélioration pourra être apportée en caractérisant plus finement le couvert prairial.

## 4-Conclusion générale

Accroître notre compréhension de la réponse des agro-écosystèmes au changement climatique est un enjeu actuel majeur pour la recherche agro-écologique. C'est avec cette ambition que cette thèse a été réalisée. Les résultats produits témoignent de l'importance du réchauffement dans le fonctionnement des prairies comme étant le facteur clé de l'impact du changement climatique. Nous avons clairement montré que le réchauffement affecte le cycle du carbone de l'écosystème prairial *via* une altération de la biomasse aérienne produite. Notre démarche a permis de mettre en évidence que le réchauffement a un effet direct puisqu'il impacte la photosynthèse et la structuration du couvert et un effet indirect *via* les modifications des ressources hydriques et nutritives. Dans notre situation, la structure de la communauté végétale a peu joué sur le fonctionnement de l'écosystème prairial principalement à cause de sa grande variabilité et de la forte plasticité des espèces peuplant notre système.

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<sup>14</sup> Pasture Simulation ; <https://www1.clermont.inra.fr/urep/modeles/pasim.htm>

Le second élément marquant de ce travail a été de montrer que le réchauffement impacte le cycle de l'azote en prairie, *via* une augmentation des pertes en azote du système sous forme de  $N_2O$  et de  $N_2$ . Cet accroissement des pertes azotées du système est directement lié à l'effet stimulant du réchauffement sur les activités enzymatiques et la communauté microbienne. Les conditions abiotiques du sol interviennent, quant à elles, de manière plus indirecte.

L'ensemble de nos données montrent que le changement climatique, *via* l'effet direct du réchauffement, affecte le cycle de l'azote en augmentant les pertes azotées du système. Ces modifications du cycle de l'azote vont impacter en retour le cycle du carbone en affectant le fonctionnement du couvert prairial. C'est donc l'augmentation des pertes azotées du système qui semble être la cause de la diminution de production de biomasse aérienne *via* une diminution de la disponibilité des ressources azotées du sol.

Notre travail apporte des éléments factuels et quantitatifs sur le fait que pour des écosystèmes semi-naturels subissant une gestion extensive sans changement d'utilisation des terres, le changement climatique a un impact fort à la fois sur les cycles du carbone et de l'azote. En l'état actuel, nos travaux permettent de prévoir qu'à terme, le changement climatique pourrait menacer la production de fourrages, la séquestration de carbone et le bilan des GES des écosystèmes semi-naturels. Il aurait donc un impact négatif sur les services écosystémiques des prairies extensives étudiées.

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## ***Annexe***

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# Effects of Warming, Summer Drought, and CO<sub>2</sub> Enrichment on Aboveground Biomass Production, Flowering Phenology, and Community Structure in an Upland Grassland Ecosystem

Juliette M. G. Bloor,<sup>1\*</sup> Patrick Pichon,<sup>1</sup> Robert Falcimagne,<sup>1</sup> Paul Leadley,<sup>2</sup> and Jean-François Soussana<sup>1</sup>

<sup>1</sup>INRA, UR874-Grassland Ecosystem Research Unit, 234 Avenue du Brézat, 63100 Clermont-Ferrand, France; <sup>2</sup>Laboratoire d'Ecologie, Systématique et Evolution, UMR CNRS 8079, Université Paris-Sud, 91405 Orsay Cedex, France

## ABSTRACT

Future climate scenarios predict simultaneous changes in environmental conditions, but the impacts of multiple climate change drivers on ecosystem structure and function remain unclear. We used a novel experimental approach to examine the responses of an upland grassland ecosystem to the 2080 climate scenario predicted for the study area (3.5°C temperature increase, 20% reduction in summer precipitation, atmospheric CO<sub>2</sub> levels of 600 ppm) over three growing seasons. We also assessed whether patterns of grassland response to a combination of climate change treatments could be forecast by ecosystem responses to single climate change drivers. Effects of climate change on aboveground production showed considerable seasonal

and interannual variation; April biomass increased in response to both warming and the simultaneous application of warming, summer drought, and CO<sub>2</sub> enrichment, whereas October biomass responses were either non-significant or negative depending on the year. Negative impacts of summer drought on production were only observed in combination with a below-average rainfall regime, and showed lagged effects on spring biomass. Elevated CO<sub>2</sub> had no significant effect on aboveground biomass during this study. Both warming and the 2080 climate change scenario were associated with a significant advance in flowering time for the dominant grass species studied. However, flowering phenology showed no significant response to either summer drought or elevated CO<sub>2</sub>. Species diversity and equitability showed no response to climate change treatments throughout this study. Overall, our data suggest that single-factor warming experiments may provide valuable information for projections of future ecosystem changes in cool temperate grasslands.

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\*Corresponding author; e-mail: juliette.bloor@clermont.inra.fr

**Key words:** biodiversity; climate change; Free Air CO<sub>2</sub> enrichment; grassland productivity; interannual variation; vegetation dynamics.

## INTRODUCTION

Current climate models predict both an increase in mean global temperatures and a change in regional patterns of rainfall as a result of rising levels of atmospheric carbon dioxide (CO<sub>2</sub>) and other greenhouse gases (IPCC 2007). Such changes could have far-reaching consequences on ecosystem structure and function, and growing evidence shows that many natural systems are already being affected by regional climate changes (for example, Parmesan and Yohe 2003; Menzel and others 2006; Post and others 2009). With future change expected, projections of ecosystem-level impacts and vulnerability of natural systems to climate change are critical for management, policy, and conservation planning. However, to accurately forecast ecosystem responses to future climatic conditions, we need a better understanding of the impacts of multiple, simultaneous global changes in the field (Luo and others 2008).

Ecosystem-scale experiments are an invaluable tool for providing the process-level information needed for improving ecosystem models in global change research (Norby and Luo 2004). During the past two decades, a number of field experiments have investigated the effects of individual climate change drivers (atmospheric CO<sub>2</sub>, temperature, rainfall) in grassland ecosystems over varying timescales (see Owensby and others 1999; Niklaus and Korner 2004; Dukes and others 2005; Grime and others 2008 and references therein). In theory, ecosystem productivity should be stimulated by elevated CO<sub>2</sub> and by warming, but shows a reduction in response to drought (Luo and others 2008). In practice, production responses to single environmental changes show considerable variability across systems and between years (Nowak and others 2004; Dukes and others 2005). For example, the extent and duration of increases in ecosystem productivity in response to CO<sub>2</sub> enrichment (driven by stimulation in leaf-level carboxylation efficiency and photosynthesis) may vary depending on plant community composition, nutrient status, and soil/plant water dynamics (Niklaus and Korner 2004; Morgan and others 2004; Luo 2007). Warming may enhance primary productivity through altered reaction kinetics, length of growing season or increases in mineralization rates and improved access to nutrients (De Boeck and others 2008), particularly in colder climates (Dukes and others 2005). Nevertheless, constraints to the warming response may include temperature stress or declines in soil moisture and nutrient availability as a result of increased

evapotranspiration (Rustad and others 2001). Variation in production responses may also be linked to phenological changes which modify patterns of soil resource acquisition and interactions between neighboring plants (Nord and Lynch 2009).

In contrast to the large body of literature available on grasslands subjected to individual climate change drivers, data on grassland responses to concurrent environmental changes are scarce. A synthesis of results from one of the most comprehensive grassland global change experiments to date, the Jasper Ridge Global Change Experiment, suggests that interactions among climate change drivers are rare and have limited influence on biomass production in the long term (Dukes and others 2005). Transient interactions between elevated CO<sub>2</sub> and rainfall on temperate grassland production may be confounded by variability in weather or nutrient availability over time (Morgan and others 2004). Furthermore, interactions between elevated CO<sub>2</sub>, air temperature, and rainfall/soil moisture do not appear to have strong effects on either grassland community diversity (Zavaleta and others 2003; Engel and others 2009) or phenology (Cleland and others 2006; Springer and Ward 2007). A recent study testing the impact of co-occurring climatic changes on artificially constructed old-field communities has shown that effects of warming and soil moisture may be more influential than elevated CO<sub>2</sub> on ecosystem structure in future environments (Engel and others 2009). However, the potential impact of multiple climatic variables on the structure and function of natural grasslands remains unclear.

Here we describe a novel climate change experiment that uses a combination of natural gradient and experimental field methods to examine the responses of an upland grassland ecosystem to single and combined climate changes in situ. Climate manipulations are based on climate change predictions for the study area for 2080 (ACACIA A2 scenario for central France, IPCC 2001), that is, a 3.5°C temperature increase, a 20% reduction in summer precipitation, and atmospheric CO<sub>2</sub> levels of 600 ppm. To our knowledge, this is the first study to explicitly test grassland responses to a published climatic scenario encompassing multiple global changes. We present the first results over three growing seasons of this ongoing, long-term climate change experiment and evaluate how warming, reduced summer rainfall, and elevated CO<sub>2</sub> affect the production, diversity, and flowering phenology in a temperate, C3 grass-dominated ecosystem. In view of the cool, wet climate in the study region

(mean annual temperature of 8.7°C, mean annual rainfall of 780 mm), we predicted that (1) warming would have a significant impact on ecosystem properties and (2) warming would underlie patterns of ecosystem response to the multifactor climate scenario tested. We hypothesized that responses to elevated CO<sub>2</sub> and reduced rainfall would be more variable, reflecting species-specific responses and interannual differences in rainfall.

## METHODS

### Study Site and Grassland Monoliths

In November 2004, 80 monoliths (0.5 × 0.5 × 0.4 m deep) were extracted from an upland grassland site located in the French Massif Central (Theix, 45°43' N, 03°01' E, 850 m a.s.l.). The climate in this area is semi-continental, with a mean annual temperature of 8.7°C and a mean annual precipitation of 780 mm. The vegetative community is dominated by grasses (*Festuca arundinacea*, *Elytrigia repens*, *Poa pratensis*, *Festuca rubra*, *Agrostis capillaris*). Other species include forbs (*Stellaria graminea*, *Veronica chamaedrys*) and legumes (*Vicia sativa*). The soil is a Cambisol developed on granite (58.6% sand, 19.4% silt, 20% clay, 2% organic matter, and C:N = 9.8) with a pH of 6.2. Prior to monolith extraction, this site had been subject to low-intensity sheep grazing (one grazing period in mid-April) with no added fertilizer for a period of 15 years (see Louault and others 2005 for further details).

Monolith extraction was carried out using a bespoke turf cutting system (following Teyssonneyre and others 2002); after extraction, the belowground zone of each monolith was encased in a stainless steel box with 81 drainage holes (1.5 cm diameter) in the bottom. At monolith extraction, 80% of the belowground biomass was estimated to be in the 0–15 cm soil layer and no roots were observed at the base of the monoliths (35–40 cm soil layer). Monoliths were left on-site to acclimate to their boxes for 6 months in specially prepared 0.4 m-deep trenches (all monoliths were flush with the soil surface and surrounded by compacted soil).

### Experimental Design and Climate Treatments

In April 2005, the botanical composition of all monoliths was determined using the point quadrat method (Teyssonneyre and others 2002), with 16 points recorded per monolith across the central 0.4 m × 0.4 m zone. Principal components anal-

ysis was used to identify five classes of monoliths according to relative abundance of dominant species (*Festuca arundinacea*, *Elytrigia repens*, *Poa pratensis*) and bare soil (data not shown). Monoliths were then lifted from their upland trenches and assigned to experimental treatments, such that each treatment included equal numbers of each vegetation class chosen at random. This ensured that the relative abundance of species at the start of the experiment was not significantly different between treatments. For logistical purposes, four monoliths were grouped together to form a single experimental unit (1 m × 1 m) and each climate treatment comprised five experimental units.

We applied four climate treatments in a replicated additive design: C (control, original site conditions), T (elevated temperature), TD (elevated temperature and summer drought), TDCO<sub>2</sub> (elevated temperature, summer drought, elevated CO<sub>2</sub>). Climate manipulations were based on climate change predictions for the area for 2080 (IPCC 2001, ACACIA A2 scenario), that is, a 3.5°C temperature increase, a 20% reduction in summer precipitation and atmospheric CO<sub>2</sub> levels of 600 ppm. Monoliths in the control treatment were left at the upland grassland site at Theix, whereas elevated temperature conditions were obtained by transplanting the remaining monoliths to a nearby lower-altitude site (Clermont Ferrand, 350 m a.s.l., mean annual temperature of 12.4°C, mean annual rainfall of 575 mm). All experimental units were placed 7 m apart to ensure that there was no risk of atmospheric contamination between CO<sub>2</sub> enriched and ambient-CO<sub>2</sub> units. Irrespective of climate treatment, all experimental units were placed in holes in the ground lined with a 0.5 m-deep layer of volcanic gravel and a 0.4 m layer of 'deep' soil (0.4–0.8 m) collected from the original monolith-extraction site. Experimental units were flush with the soil surface and surrounded by compacted soil collected from the original study site. This procedure was used to minimize differences in nutrient transfer/drainage between the experimental units and their surrounding soil.

Given that the rainfall regime at the control site corresponded to +18.3 ± 4.1 mm per month on average compared with the lowland site, rainfall at the lowland site was monitored and monoliths were irrigated weekly when necessary to equalize water inputs in C and T treatments. Summer drought in the TD treatment was achieved by reduced levels of supplemental watering during June, July, and August. In the event of the occasional heavy summer rainstorm at the lowland site, transparent plastic rain-out shelters were set up

over the relevant experimental units for short periods (24–48 h). Rain-out shelters were constructed using a sloping wooden frame and placed at a height of approximately 0.8 m to allow near-surface air exchange; no significant temperature difference was observed under rain-out shelters during rain manipulation periods.

Mini-FACE (Free Air CO<sub>2</sub> Enrichment) techniques were used to establish the elevated CO<sub>2</sub> treatment following Miglietta and others 2001. In brief, we set up an automated control system and 1.5 m-diameter rings (corrugated polyethylene vent pipes) around each TDCO<sub>2</sub> experimental unit, through which CO<sub>2</sub>-enriched air was released. The maximum distance between the control system and the mini-FACE rings was 20 m. The CO<sub>2</sub> target concentration of 600 ppm was monitored and maintained during daylight hours via input/output signals at the control system (see Teyssonneyre and others 2002 for full details); to ensure delivery of enriched air to the top of the plant canopy, the height of the rings above soil level was adjusted over the course of the growing season. To avoid confounding effects of increased air flow over the plant canopy in the TDCO<sub>2</sub> treatment, 1.5 m-diameter rings blowing ambient air were set up around all the remaining experimental units (C, T and TD treatments). CO<sub>2</sub> fumigation was fully operational from 17 June 2005 and was only interrupted in the rare event of snow cover or maximum daily temperatures below 0°C.

### Sward Management and Data Acquisition

Once all experimental units were in place (27th April 2005), grass monoliths were cut to a height of 5 cm;  $t_0$  biomass data were derived from a central 0.4 × 0.4 m zone per monolith and the remaining 5 cm-wide strip around the perimeter of each monolith was discarded to avoid edge effects. Subsequent cuts were carried out at 6-monthly intervals, that is, late October and late April each year through to 2007. These harvests correspond roughly to the start and end of the plant growing season in an average year at the upland control site, peak biomass being obtained around mid-June. During the study period, April harvests occurred 2–3 weeks after re-greening and initiation of vegetative growth at the control site. At each cut, a 25–45 g subsample of freshly harvested above-ground biomass from each monolith was sorted into green and senescent material; this subsample corresponded to approximately half of the harvested biomass per monolith ( $49 \pm 2.2\%$  on average). Green biomass material was sorted to species

and all plant biomass samples were then dried at 60°C for 48 h prior to weighing. Species biomass samples were used to derive Shannon–Weaver diversity and equitability indices (Kent and Coker 1992).

Flowering of all grass species in each monolith was recorded from mid-April through to mid-July in both 2006 and 2007; during these monitoring periods, the number of inflorescences with exposed stamens and/or styles was recorded three times a week. These observations allowed calculation of the flowering onset date (time to first flowering = number of days from 1 January) per species per experimental unit.

Climate measurements were made using a Campbell Scientific automatic weather station with half-hourly measurements logged to a CRX-10 data logger (Campbell Scientific Inc., Utah, USA) at both the lowland and upland sites throughout the experimental period.

### Statistical Analysis

Treatment differences in biomass, proportional contributions of individual species to community biomass and phenology were analyzed using mixed model repeated measures analysis of variance (ANOVA) and mean values per variable per experimental unit. We determined the effects of individual climate change drivers (temperature, drought, CO<sub>2</sub>) using specific, planned orthogonal contrasts and the MS<sub>error</sub> terms from the repeated measures ANOVA analyses previously described (following Gilligan 1986): effects of warming were examined by comparing the C and T treatment; effects of summer drought were examined by comparing T and TD; effects of elevated CO<sub>2</sub> were investigated by the TD versus TDCO<sub>2</sub> comparison; effects of simultaneous application of warming, summer drought, and CO<sub>2</sub> enrichment (2080 climate scenario) were assessed by comparing C with TDCO<sub>2</sub>. Where necessary, data were transformed prior to analysis to conform to assumptions of normality and homogeneity of variances; statistical analysis was carried out using Statgraphics Plus 4.1 (Statistical Graphics Corp., Rockville, Maryland, USA).

Floristic changes through time were analyzed using a non-metric multidimensional scaling (NMS) approach in PC-ORD 5.0 (MjM Software Design, Gleneden Beach, Oregon, USA). Species contributing less than 1% of the total biomass throughout the study were not included in the NMS data matrix because (i) the objective was to identify changes in dominant species likely to

contribute to the functional properties of the study grassland and (ii) rare species may enhance noise and instability in the ordination results (McCune and Grace 2002). The NMS analysis was run with Sørensen's index and two dimensions (determined by a preliminary step-down procedure as recommended by McCune and Grace 2002). Subsequent to NMS analysis, the linear distance between resampled experimental units in NMS ordination space was used as an index of plant compositional change following McCune and Grace (2002).

**RESULTS**

**Meteorological Conditions and Climate Treatments**

Precipitation at the control site varied from substantially below average in 2005 to slightly above average in 2006 and considerably above average in 2007 (Figure 1). Air temperatures were slightly above average during the growing season in 2005 and 2006, distinctly above average during the summer of 2006 but below average during the summer of 2007 (Figure 1). In addition, 2005 and 2006 were characterized by severe winters whereas 2007 was characterized by a warmer winter and spring period than average.

During the experimental period, differences in mean monthly temperatures between the control and elevated temperature treatments ranged from 1.8 to 4.6°C (average difference  $3.46 \pm 0.15^\circ\text{C}$ , Figure 2). Differences in precipitation between C and T treatments were effectively minimized by supplemental watering (mean annual difference  $21.2 \pm 5.48$  mm, <3% of total rainfall). Differences in summer rainfall between the T and TD treatments reached the 20% target and ranged between 21 and 89.6 mm from 2005 to 2007 (mean monthly rainfall difference  $20.5 \pm 1.42\%$ ). Mean daily CO<sub>2</sub> values in the TDCO2 treatment from May 2005 to December 2007 were  $588 \pm 2.1$  ppm compared with  $395 \pm 3.2$  ppm recorded in ambient CO<sub>2</sub> conditions during

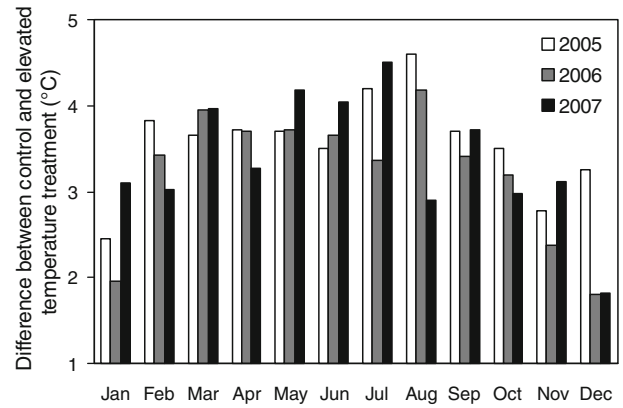


Figure 2. Mean monthly temperature difference recorded between the lowland, elevated temperature site and the upland, control site during this study.

the same period. No significant differences in ambient CO<sub>2</sub> values or global radiation were observed between the control, upland site, and the lowland site during the study (data not shown).

**Aboveground Biomass Production**

At the start of the study in April 2005 (t<sub>0</sub>), total biomass above 5 cm averaged 70 g m<sup>-2</sup> across experimental units and climate treatments (Figure 3). Over the following three growing seasons, aboveground biomass in individual experimental units ranged from 87.0 to 269.1 g m<sup>-2</sup> for harvests in April and from 184.2 to 543.0 g m<sup>-2</sup> for harvests in October (Figure 3). Aboveground biomass production averaged across treatments showed no significant variation between 2006 and 2007 (*P* > 0.1 for both April and October harvests).

Effects of individual climate change drivers on total aboveground biomass varied depending on both season and year of measurement (Table 1; Figure 3). In 2005, neither April nor October biomass showed any significant response to single climate drivers (*P* > 0.1). In April 2006, 1 year after the start of experimental treatments, warming had a

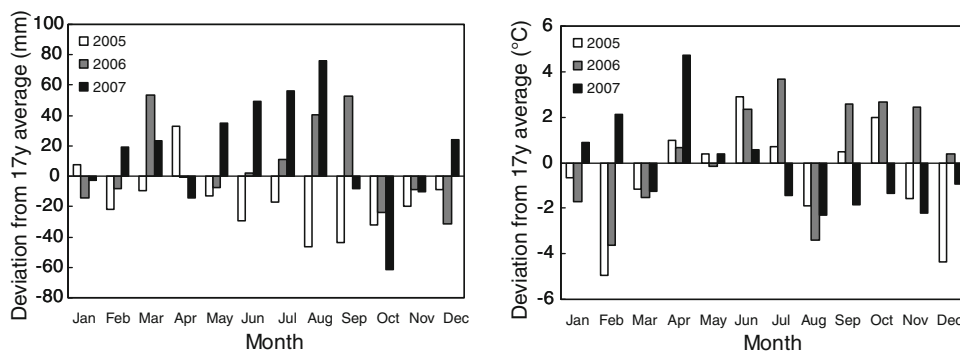
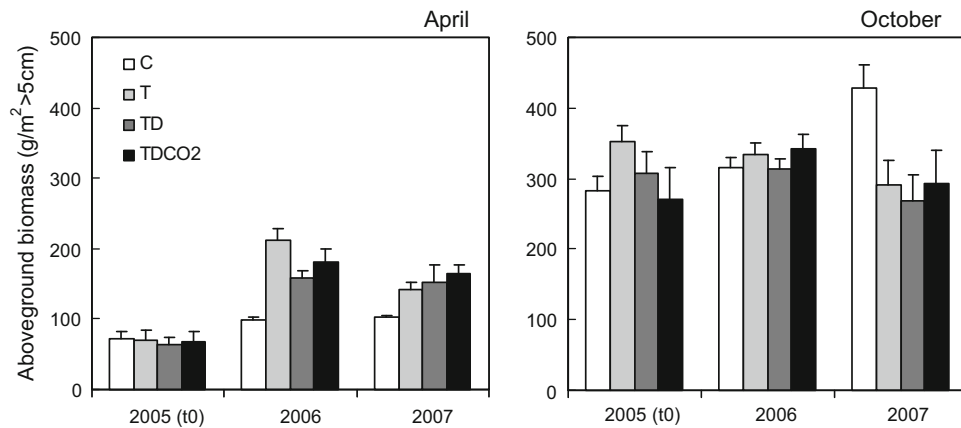


Figure 1. Monthly rainfall and mean monthly temperatures recorded during this study for the control study site (2005–2007) compared with the 17-year average.





**Figure 3.** Effects of climate manipulations on spring and autumn aboveground biomass. Treatment codes are given by: C control; T elevated temperature (+3.5°C); TD elevated temperature and summer drought (−20%); TDCO2 elevated temperature, summer drought, and CO<sub>2</sub> enrichment (+200 ppm). Means and standard errors are presented per experimental unit ( $n = 5$ ).

**Table 1.** Effects of Climate Treatment on Aboveground Biomass Harvested in April and October

(a) April				(b) October			
Effect	df	F	P	Effect	df	F	P
Climate treatment	3	8.74	**	Climate treatment	3	1.11	NS
Error	16			Error	16		
Within subjects (units)				Within subjects (units)			
Year	2	55.3	***	Year	2	0.8	NS
Climate × year	6	4.78	**	Climate × year	6	3.79	**
Error	32			Error	32		

Results of repeated measures analysis of variance (ANOVA), using years as a repeated measures factor. NS not significant. \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ .

significant positive effect on aboveground biomass (C versus T treatment,  $F_{1,16} = 36.2$ ,  $P < 0.001$ , 119% biomass increase) whereas the summer drought treatment was associated with a significant negative effect on biomass (T versus TD treatment,  $F_{1,16} = 8.1$ ,  $P < 0.05$ , 25% spring biomass reduction). In 2007, however, neither warming nor summer drought had any significant effect on April biomass, although a tendency toward increased biomass production in response to warming was observed (Figure 3). Elevated CO<sub>2</sub> had no significant effect on April aboveground biomass in either 2006 or 2007 (TD versus TDCO2,  $P > 0.1$ ), although there was a tendency for increased spring biomass production in response to CO<sub>2</sub> in 2006 (Figure 3). Nevertheless, global climate change (C versus TDCO2) was associated with a significant increase in April biomass production in both 2006 and 2007 (+88%,  $F_{1,16} = 20.3$ ,  $P < 0.001$  and +60%,  $F_{1,16} = 9.2$ ,  $P < 0.05$ , respectively).

In contrast to pronounced spring biomass responses to climate change drivers, responses of autumn biomass were more limited (Figure 3). Neither summer drought nor CO<sub>2</sub> enrichment had any significant effect on biomass harvested in October throughout the experiment (no significant differences for either T versus TD or TD versus TDCO2 comparisons, respectively). However, three growing seasons after the start of the experiment (that is, October 2007), warming had a significant negative effect on October biomass (C versus T,  $F_{1,16} = 7.8$ ,  $P < 0.05$ , 31% biomass reduction). Simultaneous application of warming, summer drought, and CO<sub>2</sub> enrichment mirrored patterns of warming on autumn biomass: in 2006, there was no significant difference between C and TDCO2 whereas in 2007, October biomass production was significantly lower in the TDCO2 compared with C treatment (−47%,  $F_{1,16} = 7.5$ ,  $P < 0.05$ ).

## Plant Community Composition

The number of species collected per monolith during April and October harvests ranged from 4 to 9 (mean of  $6.7 \pm 0.11$  species) during the study period, and showed no significant variation between 2006 and 2007 ( $P > 0.1$  for both April and October harvests). Grass species dominated the experimental plant communities, accounting for more than 90% of the biomass in all experimental units (average of  $93.5 \pm 0.71\%$ ). The proportion of graminoids to non-graminoids in the experimental units remained stable between years. In contrast, the proportion of legumes (*Vicia sativa*) in the non-graminoid fraction increased significantly between 2006 and 2007 for the spring harvests (mean abundance of  $0.2 \pm 0.09\%$  and  $2.5 \pm 0.71\%$  in 2006 and 2007, respectively,  $F_{2,16} = 8.59$ ,  $P < 0.05$ ).

Temporal patterns in graminoid floristic composition were more marked for April harvests than for October harvests; a significant two-dimensional NMS ordination was obtained for April harvests (Figure 4), whereas ordination of October harvests did not differ significantly from random. The cumulative proportion of the variation explained by the April ordination was 0.87 (0.34 and 0.52 for axes 1 and 2, respectively), with a final stress of 16.08 indicating a satisfactory ordination result. The first NMS axis represented a time gradient, with all experimental units showing a transition parallel to the first ordination axis (Figure 4). However, line segments connecting the resampled experimental units showed considerable variation, and there was no significant difference in the amounts of change between climate treatments. Axis 1 of the NMS ordination was strongly correlated with *Elytrygia repens* ( $r = -0.87$ ) and *Festuca rubra* ( $r = 0.65$ ), whereas axis 2 was strongly correlated with *Festuca arundinacea* ( $r = -0.69$ ) and *Arrhenatherum elatius* ( $r = 0.92$ ).

Large heterogeneity in dominant plant distributions made it difficult to detect effects of climate change drivers on plant community structure during the study period (Table 2). In 2006, no significant treatment differences were detected on the abundance of individual species, species diversity (Shannon–Weaver) or equitability for both April and October harvests ( $P > 0.1$  in all cases). In April 2007, the relative contribution of *Agrostis capillaris* to community biomass decreased in the drought treatment but showed a significant increase in response to elevated CO<sub>2</sub> ( $-50\%$  in TD compared with T,  $F_{1,16} = 16.1$ ,  $P < 0.01$  and  $+182\%$  for TDCO2 versus TD,  $F_{1,16} = 11.7$ ,  $P < 0.01$ ,

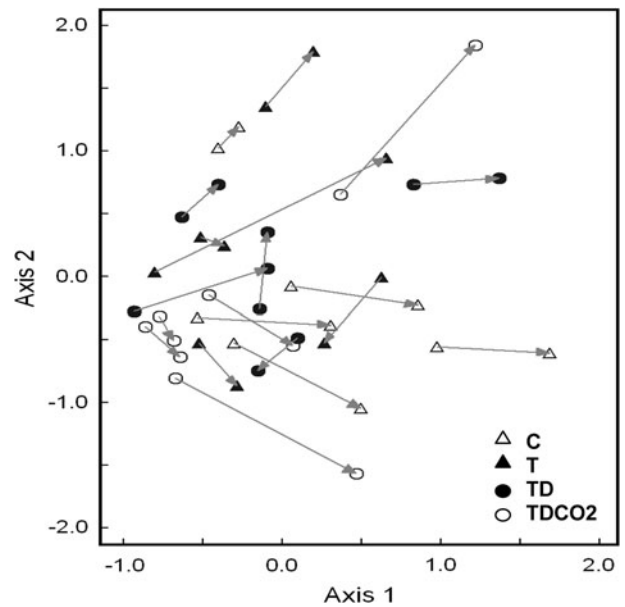


Figure 4. Results of NMS ordination based on grass species relative abundances in April harvests. Plant compositional change from 2006 to 2007 is represented by lines connecting resampled experimental units; the graph has been rotated such that axis 1 corresponds to a time gradient. Treatment codes are given by: C control; T elevated temperature ( $+3.5^{\circ}\text{C}$ ); TD elevated temperature and summer drought ( $-20\%$ ); TDCO2 elevated temperature, summer drought, and CO<sub>2</sub> enrichment ( $+200$  ppm).

respectively). A decrease in the relative proportion of *Trisetum flavescens* was observed in response to warming in April 2007 (C versus T,  $-75\%$ ,  $F_{1,16} = 11.3$ ,  $P < 0.01$ ). Simultaneous application of warming, drought, and CO<sub>2</sub> was also associated with a significant decline in *Trisetum flavescens* abundance in April 2007 (C versus TDCO2,  $-68\%$ ,  $F_{1,16} = 9.32$ ,  $P < 0.05$ ). In October 2007, experimental warming was associated with a significant decrease in the relative contribution of *Dactylis glomerata* to community biomass (C versus T,  $-75\%$ ,  $F_{1,16} = 6.12$ ,  $P < 0.05$ ). Nevertheless, species diversity and equitability showed no significant climate treatment effects in either April or October 2007.

## Flowering Phenology

Five grass species were found flowering in all the experimental units during the study (Table 3). Flowering commencement date averaged across treatment and species did not vary between years (day  $134.4 \pm 1.3$  and  $134.5 \pm 0.6$  in 2006 and 2007, respectively), despite a marked difference in the corresponding thermal sum between years (933 degree-days in 2006 compared with 1327

**Table 2.** Mean Species Contributions (%) to Community Biomass in Experimental Climate Treatments

Species	Year	Climate treatment			
		C	T	TD	TDCO2
<i>Agrostis capillaris</i>	2006	4.63 (0.86)	6.85 (1.98)	4.70 (0.90)	7.15 (2.28)
	2007	6.66 (2.78)	5.94 (1.19)	3.39 (0.68)	7.42 (1.52)
<i>Arrhenatherum elatius</i>	2006	5.48 (2.05)	13.6 (2.45)	9.19 (3.07)	5.36 (2.72)
	2007	5.73 (2.71)	18.3 (5.74)	13.7 (3.86)	5.18 (3.29)
<i>Festuca arundinacea</i>	2006	19.8 (5.26)	18.6 (4.25)	12.1 (3.06)	14.9 (5.90)
	2007	23.6 (6.40)	22.8 (4.81)	16.5 (3.98)	25.6 (7.66)
<i>Festuca rubra</i>	2006	11.0 (3.80)	2.20 (1.72)	5.06 (1.59)	6.11 (2.12)
	2007	13.1 (4.52)	4.72 (2.56)	3.67 (0.92)	6.10 (2.59)
<i>Dactylis glomerata</i>	2006	4.69 (1.18)	2.87 (0.72)	2.46 (1.40)	3.90 (0.79)
	2007	10.2 (3.16)	2.52 (0.80)	2.12 (1.27)	4.29 (1.08)
<i>Elytrygia repens</i>	2006	37.7 (5.38)	40.1 (5.77)	39.5 (5.76)	42.2 (6.72)
	2007	23.9 (5.70)	26.7 (6.02)	31.8 (5.98)	30.8 (6.66)
<i>Trisetum flavescens</i>	2006	1.87 (0.76)	1.08 (0.66)	1.45 (0.53)	1.01 (0.37)
	2007	2.04 (0.76)	0.95 (0.21)	0.88 (0.20)	1.05 (0.45)
<i>Poa pratensis</i>	2006	10.8 (3.32)	12.3 (2.68)	21.8 (6.26)	13.5 (3.43)
	2007	9.42 (2.63)	11.3 (2.87)	19.1 (4.79)	8.86 (3.18)

Percentage species contributions of eight dominant grass species averaged across spring and autumn harvests for each experimental unit per year; treatment means and standard errors are shown ( $n = 5$ ).

Treatment codes are given by: C control; T elevated temperature; TD elevated temperature and summer drought; TDCO2 elevated temperature, summer drought, and CO<sub>2</sub> enrichment.

**Table 3.** Effects of Warming, Summer Drought, Elevated CO<sub>2</sub> and Simultaneous Application of Climate Drivers on the Onset of Species Flowering

Species	Year	Warming (C versus T)		Summer drought (T versus TD)		Elevated CO <sub>2</sub> (TD versus TDCO2)		2080 Climate scenario (C versus TDCO2)	
		F	P	F	P	F	P	F	P
<i>Agrostis capillaris</i>	2006	16.5	**	0.01	NS	0.44	NS	11.46	**
	2007	5.15	MS	1.90	NS	0.94	NS	3.46	NS
<i>Festuca arundinacea</i>	2006	21.8	***	0.01	NS	0.37	NS	16.74	**
	2007	5.23	MS	0.04	NS	0.01	NS	5.68	MS
<i>Festuca rubra</i>	2006	0.71	NS	0.09	NS	1.23	NS	5.08	MS
	2007	1.75	NS	1.30	NS	0.29	NS	0.52	NS
<i>Poa pratensis</i>	2006	3.36	NS	0.05	NS	0.29	NS	4.58	MS
	2007	1.02	NS	1.68	NS	1.84	NS	1.15	NS
<i>Trisetum flavescens</i>	2006	15.07	**	0.03	NS	0.20	NS	20.21	***
	2007	0.09	NS	0.03	NS	0.85	NS	0.45	NS

Values shown are from planned F-ratio tests following Gilligan (1986).

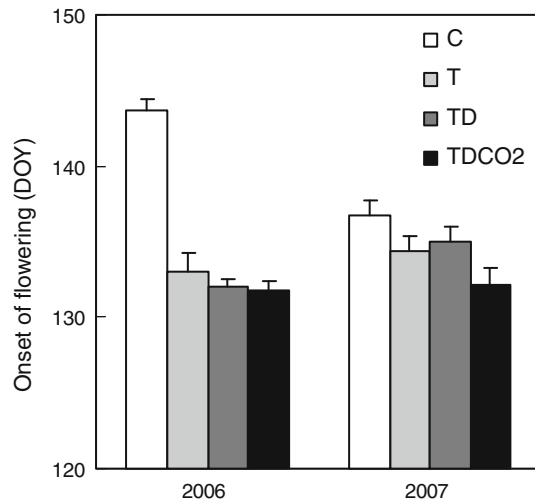
NS not significant. <sup>MS</sup>  $P < 0.1$ , \* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$ ;  $df_{error} = 16$ . Significant results indicate earlier onset of flowering in all cases.

Treatment codes are given by: C control; T elevated temperature; TD elevated temperature and summer drought; TDCO2 elevated temperature, summer drought, and CO<sub>2</sub> enrichment.

degree-days in 2007). However, effects of climate treatments on start of flowering differed between years (significant climate  $\times$  year interaction,  $F_{3,16} = 16.4$ , and  $P < 0.001$ ). In 2006, both experimental warming and the simultaneous application of warming, drought and CO<sub>2</sub> significantly advanced the onset of flowering across species

(T compared to the C,  $-11.4$  days on average,  $F_{1,16} = 46.8$ ,  $P < 0.001$ ; TDCO2 compared with C,  $-12.3$  days on average,  $F_{1,16} = 59.0$ ,  $P < 0.001$ ). In 2007, flowering dates averaged across species showed no significant response to warming alone. Nevertheless, simultaneous application of warming, drought and CO<sub>2</sub> was associated with a





**Figure 5.** Effects of climate manipulations on flowering commencement date averaged across grass species. Treatment codes are given by: *C* control; *T* elevated temperature (+3.5°C); *TD* elevated temperature and summer drought (−20%); *TDCO2* elevated temperature, summer drought, and CO<sub>2</sub> enrichment (+200 ppm). Means and standard errors are presented ( $n = 5$ ).

significant advance in flowering dates across species (4.6 days earlier on average in TDCO2 compared with *C*,  $F_{1,16} = 8.8$ ,  $P < 0.05$ ). Neither summer drought nor elevated CO<sub>2</sub> alone had any effect on flowering dates during the study (Figure 5).

In agreement with the average response, flowering commencement dates at the species level showed stronger responses to elevated temperature than to the other single climate change drivers. First flowering dates were significantly affected by warming for three out of five species in 2006 (Table 3); onset of flowering was advanced by an average of 10.9 days for *A. capillaris*, 13.9 days for *T. flavescens*, and 14.5 days for *F. arundinaceae*. In 2007, *A. capillaris* and *F. arundinaceae* also showed a marginally significant advance in flowering commencement date in response to warming. However, none of the species studied showed any significant change in flowering commencement dates in response to either summer drought or elevated CO<sub>2</sub> throughout the study (Table 3). Effects of combined warming, drought, and CO<sub>2</sub> on species-level flowering dates varied depending on the year. In 2006, concurrent climate change drivers advanced the onset of flowering for all species monitored (significant advance in flowering dates for three out of five species and a marginally significant advance for the remaining two species in TDCO2 compared to *C*, Table 3). In 2007,

simultaneous application of warming, drought, and CO<sub>2</sub> was associated with a marginally significant advance in flowering date for *F. arundinaceae* alone.

Despite interspecific variation observed in response to climate manipulations, differences in flowering onset date between species were maintained; species ranking according to flowering commencement dates produced a consistent pattern across years irrespective of experimental treatment (*P. pratensis* < *F. rubra* < *F. arundinaceae* < *T. flavescens* < *A. capillaris*). Furthermore, climate treatments had little effect on the variability of flowering onset dates between species (coefficients of variation across treatments ranged from 6.3 to 8.5 in 2006 and from 5.7 to 6.0 in 2007).

## DISCUSSION

### Effects of Climate Change on Biomass and Plant Community Structure

Climate-driven changes in grassland productivity could have serious consequences for the distribution and profitability of pastoral agriculture (Grime and others 2000; Tubiello and others 2007). In line with our predictions, patterns of biomass response to the 2080 climate scenario showed a close correspondence with the biomass responses to warming alone. Both warming and combined climate change drivers had a positive effect on April biomass (that is, production over the late autumn-early spring period), although the magnitude of change varied between years. In contrast, impacts of both warming and the 2080 climate scenario on October biomass (that is, production over the late spring-early autumn period) were either non-significant or negative depending on the year.

Although experimental warming tends to have a positive effect on production across ecosystems, grassland experiments that show clear increases in productivity in response to warming are relatively rare (Rustad and others 2001; Dukes and others 2005; Grime and others 2008). Our results support the idea that positive effects of warming are more prevalent in colder environments because (i) we found that increases in production in response to warming were restricted to the coldest part of the year, and (ii) smaller warming-induced increases in production were observed during the milder winter of 2007. Stimulatory effects of warming at the start of the growing season may be reversed in the summer due to soil drying and decreased soil N availability (Luo 2007); this may explain the lack of warming response found for October biomass in 2006. However, such a mechanism seems unlikely

to account for the patterns of October production following the cool, wet summer of 2007. We do not rule out the possibility that climate effects on aboveground grassland production were influenced by carryover effects from previous years (Sherry and others 2008) or by changes in surface litter production (Zhou and others 2009).

Precipitation is known to be a key driver of grassland productivity; as a result, grasslands often exhibit strong and rapid functional responses to experimentally altered rainfall patterns (Grime and others 2008; Fay and others 2003; Dukes and others 2005). Nevertheless, we only found a significant negative effect of summer drought on April biomass in the second year of study (2006). Given the timing of the drought treatment, it seems reasonable to suppose that this represents a lagged drought effect from the previous summer. Unlike in 2006 and 2007, the drought treatment imposed in 2005 exacerbated a below-average summer rainfall regime. Our study, therefore, suggests that grassland production responses to drought reflect an interaction between rainfall manipulation treatment and the ambient rainfall regime. Why didn't biomass production show a more rapid response to summer drought (that is, effects on October biomass)? Species-level physiological plasticity may have buffered community-level responses to drought in the short term (Chaves and others 2003). Alternatively, the observed lag effects could have been mediated by soil moisture recharge from deep soil layers (Sherry and others 2008). Non-significant differences in soil moisture for the 0–20 cm soil layer between the T and TD treatments during the summer of 2005 indicate that winter/spring rainfall may have been sufficient to drive summer growth in our drought treatments (data not shown). However, we were unable to determine whether soil water availability was depleted in the TD treatment during autumn and winter 2005 due to problems with soil moisture probes. No significant differences in soil moisture content were observed between T and TD from spring 2006 until the end of the study (R. Pilon unpublished data).

Unlike warming or summer drought, elevated CO<sub>2</sub> had no significant effect on aboveground production at any time during our study. Such a lack of sensitivity to CO<sub>2</sub> is somewhat unexpected given the potential for improved plant water relations under elevated CO<sub>2</sub> (but see Potvin and Vasseur 1997; Dukes and others 2005). This could partly reflect the relatively high rainfall regime and non-limiting water conditions during much of the study period. Increases in grassland productivity in response to CO<sub>2</sub> are often more prevalent in years

of water stress (Owensby and others 1999; Niklaus and Korner 2004; Morgan and others 2004), and it is notable that we observed a tendency for enhanced production in response to CO<sub>2</sub> during the same harvest that we detected significant effects of summer drought (April 2006). However, the CO<sub>2</sub>-induced biomass responses in this unfertilized and relatively unproductive grassland may also have been constrained by nutrient limitation; N limitation commonly leads to smaller productivity enhancement under elevated CO<sub>2</sub> (reviewed by Reich and others 2006). Indeed, nitrogen nutrition indices (biomass production described as a function of leaf N content; Lemaire and Gastal 1997) provided some evidence of N limitation across all treatments throughout the study (nitrogen nutrition values of 60–80 rather than 100 which represents non-limiting N conditions).

Patterns of biomass response to climate change may be mediated by shifts in the relative contribution of individual species to community biomass (Leadley and others 1999; Engel and others 2009). We found some indication of climate-induced changes in species abundances, but there were no consistent patterns across species, season, or climatic treatment. Furthermore, neither global climate change nor single climate change drivers had any significant effect on species diversity or equitability. The low sensitivity of community structure reported here corroborates the results of other studies of grass species subjected to multiple climate change drivers (Zavaleta and others 2003; Grime and others 2008; Engel and others 2009), although more pronounced diversity responses have been detected in response to elevated CO<sub>2</sub> alone (Owensby and others 1993; Potvin and Vasseur 1997; Teyssonneyre and others 2002; Niklaus and Korner 2004). Our findings provide support for the idea that resistance of grassland composition to climate change may be higher in unproductive, mature communities (Grime and others 2000) and in low diversity communities (Niklaus and others 2001; De Boeck and others 2008). Stability in community structure may also be mediated by phenotypic plasticity in some species (Owensby and others 1999). As yet, the role of plant traits in determining grassland responses to climate change remains to be tested.

## Phenological Responses to Climate Change

Timing of flowering is a critical aspect of a plant lifecycle, with implications for pollination success, fruit ripening, and seed dispersal. Previous studies in herbaceous communities indicate that rising

temperatures generally promote earlier flowering, and that the shift in spring flowering for grassland species ranges from 1.3 to 11 days advancement per degree of warming (Fitter and Fitter 2002; Dunne and others 2003; Cleland and others 2006; Hovenden and others 2008). In this study, experimental warming resulted in an acceleration in flowering of 3.2 days per degree of warming across grass species in 2006. However, warming had very little impact on either the average flowering response or the flowering responses of individual species in 2007.

Interannual variation in flowering responses to warming may reflect interactions between photoperiod and ambient temperatures between years, and it has been suggested that warming-induced increases in developmental rates may be constrained by other abiotic factors beyond a certain temperature threshold (Hovenden and others 2008). At our study sites, the winter and early spring period of 2007 was characterized by unusually warm ambient temperatures (highlighted by the large difference in thermal sum between 2006 and 2007 at time of flowering). Given that we found very close agreement in mean flowering time in warmed plots between years (day  $131.9 \pm 2.2$  and  $133.5 \pm 1.9$  in 2006 and 2007, respectively), our results support the idea that flowering phenology shows a non-linear response to temperature, and that day length is an important environmental cue for grass flower production in this upland ecosystem.

Patterns of flowering response to simultaneous application of warming, drought, and CO<sub>2</sub> mirrored variation in flowering times in response to warming at both the species and community level; neither elevated CO<sub>2</sub> nor summer drought had any effect on flowering phenology in this study. The lack of response to summer drought treatment is perhaps unsurprising given that the drought period coincided with the end of the grass flowering period each year. Our results are consistent with other studies on flowering in enriched CO<sub>2</sub> environments which show either no response or no clear pattern of response of flowering times to CO<sub>2</sub> (reviewed by Springer and Ward 2007; Hovenden and others 2008). Such variation in observed flowering time responses to elevated CO<sub>2</sub> has been attributed in part to differences in experimental growing conditions among studies, and to plasticity in phenology across wild species (Springer and Ward 2007).

Shifts in phenology in response to climate change have previously been shown to decrease the phenological complementarity between coexisting forbs and grasses, with ramifications for species coexistence and ecosystem processes

(Cleland and others 2006). However, we found little impact of climate change treatments on the phenological overlap among grass species in this study. These findings may in part reflect the low variability in flowering times for species within the same functional group. Of course, we have only addressed one aspect of flowering phenology in this study, and duration of flowering is likely to be equally as important in determining phenological complementarity among species. To date, studies of climate change on flowering duration in grasslands yield conflicting results (Dunne and others 2003; Hovenden and others 2008) and further study is required in this area.

## CONCLUSION

During the experimental period, we observed significant seasonal and interannual variation in grassland responses to climate treatments. Overall, we found that production and phenology showed greater responses than grassland community structure to both single and combined climate change drivers. In line with our predictions, warming was the single most important climate driver on ecosystem properties. Patterns of ecosystem response to simultaneous application of warming, drought, and CO<sub>2</sub> (2080 climate scenario) mirrored those in response to warming alone, suggesting that results from single factor warming experiments may provide valuable information for forecasting ecosystem changes in this study system. Future studies should address the impact of climate change on plant functional traits in unproductive grasslands, and determine whether the results obtained here over three growing seasons can be generalized in the longer term.

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