

UNIVERSIDADE FEDERAL DO PARANÁ

ANDRÉ FAÉ GIOSTRI

PRODUCTION AND ENVIROMENTAL SERVICES OF INTEGRATED CROP  
LIVESTOCK SYSTEMS

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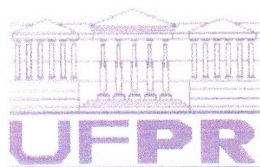
ANDRÉ FAÉ GIOSTRI

PRODUCTION AND ENVIRONMENTAL SERVICES OF INTEGRATED CROP  
LIVESTOCK SYSTEMS

Thesis submitted to the Course of Post Graduation in Agronomy, Concentration Area in Plant Production - Agronomy, Department of Agricultural Sciences, Federal University of Paraná, as partial requisite to obtain the title of Doctor in Science.

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UNIVERSIDADE FEDERAL DO PARANÁ  
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PROGRAMA DE PÓS-GRADUAÇÃO EM  
AGRONOMIA - PRODUÇÃO VEGETAL





## PARECER

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Agronomia - Produção Vegetal, reuniram-se para realizar a arguição da Tese de DOUTORADO, apresentada pelo candidato **ANDRÉ FAÉ GIOSTRI**, sob o título "**PRODUCTION AND ENVIRONMENTAL SERVICES OF INTEGRATED CROP LIVESTOCK SYSTEMS**", para obtenção do grau de Doutor em Ciências do Programa de Pós-Graduação em Agronomia - Produção Vegetal do Setor de Ciências Agrárias da Universidade Federal do Paraná.


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## RESUMO

Sistemas diversificados de produção agropecuária, tais como sistemas integrados de produção agropecuário (PISA) são uma alternativa para o aumento produção e melhorias nos ecossistemas. As características distintas destes sistemas de produção são explorar sinergias e propriedades emergentes de interações no compartimentos solo-planta-animal-atmosfera em áreas que integram os PISA, também fornecer interações ecológicas entre os diferentes ecossistemas agrícolas, como a redução da degradação química e física do solo, aumentando a matéria orgânica do solo, preservação dos recursos naturais e benefícios ambientais, como a redução das emissões de gases com efeito de estufa. Assim, a utilização da interação em PISA é fundamental para alcançar sucesso, como resultado final, para aumentar o rendimento e a sustentabilidade ecológica. O primeiro capítulo avalia o efeito árvores de um PISA sobre a qualidade e a produção de seis espécies forrageiras *Axonopus catharinensis*, *Urechloa brizantha* cv. Marandu, capim-mombaça cv. Aruana, *Hemarthria altíssima* cv. Florida, *Cynodon* spp. híbrido Tifton 85 e *Paspalum notatum* cv. Pensacola. O segundo capítulo avalia as emissões de  $N_2O$  em um PISA com rotações de longo prazo: pastagem cortada seguida de três anos com rotações agrícolas . O terceiro capítulo aborda a questão do  $N_2O$  em sistemas com diferentes manejos de pastagens (cortada vs. pastejada). Concluiu-se que as espécies de forrageiras apresentaram menor rendimento produção no PISA estudado, devido ao efeito do sombreamento das árvores. A adubação nitrogenada aumentou o valor nutritivo e produção de matéria seca das forragens. Os resultados da emissão de  $N_2O$  mostraram que aração do solo aumenta a emissão de  $N_2O$  do solo. A pastagem cortada também contribui para o aumento da emissão de  $N_2O$  solo, comparando com a forragem pastejada.

Palavras chave:  $N_2O$ , rotação de culturas, forragem, manejo de pastagem, gases do efeito estufa.

## ABSTRACT

Diversified agricultural systems such as integrated crop livestock systems are an alternative for achieving production and ecosystem services. The distinguishing characteristics of these production systems are that they are designed to exploit synergisms and emergent properties of interactions in the compartments soil-plant-animal-atmosphere on areas that integrate crop and livestock production systems (ICLS). ICLS also can provide opportunities to capture ecological interactions among different land use systems to make agricultural ecosystems more efficient at reducing soil chemical and physical degradation, increasing soil organic matter, enhancing biodiversity and preserving natural resources and environmental benefits as reducing greenhouse gas emission. Thus, the use of the interaction in ICLS is key to achieving success, as final result to increase ecological sustainability and yield. The first chapter evaluates the trees effect of an ICLS on the quality and production of six forage species *Axonopus catharinensis*, *Uruclioa brizantha* cv. *Marandu*, *Megathyrsus maximus* cv. *Aruana*, *Hemarthria altissima* cv. *Florida*, *Cynodon* spp. hybrid *Tifton 85* and *Paspalum notatum* cv. *Pensacola*. The second chapter assesses the emission of  $N_2O$  on an ICLS with long-term rotations: mowed pasture and three years crop rotations. The third chapter addresses the issue of  $N_2O$  in systems with different pasture management (mowed vs. grazed). It was concluded that the forage species showed lower production in the ICLS, due to trees effect. Nitrogen increased the forage nutritional value and dry matter yield. The results of  $N_2O$  emission showed that plowing increases the soil  $N_2O$  emission and the mowed grassland also contributes to the increase in the soil  $N_2O$  emission, compare to grazing.

Key words:  $N_2O$ , crop rotation, forages, pasture management, greenhouse gas.

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## 1. INTRODUCTION

Diversified systems such as crop-livestock systems appear to be an interesting alternative and path forward for agricultural development in the face of climate change and volatility of commodity and input prices.

The use of integrated crop livestock production systems (ICLS) is an alternative for achieving sustainability. The distinguishing characteristics of these production systems are that they are designed to exploit synergisms and emergent properties of interactions in the compartments soil-plant-animal-atmosphere areas and integrate crop and livestock production (Moraes et al., 2013).

ICLS also can provide opportunities to capture ecological interactions among different land use systems to make agricultural ecosystems more efficient at reducing soil chemical and physical degradation, increasing soil organic matter, enhancing biodiversity and preserving natural resources and environmental benefits as reducing greenhouse gas emission (Smith et al., 2007).

Thus, the use of the interaction in ICLS is key to achieving success, as final result to increase ecological sustainability and yield. In this sense, the interactions must be planned in different spatial-temporal scales and in landscape level to cover crop and animal production and ecological sustentability. Although is necessary the knowledge to understand the interaction effect between the biotic and abiotic factors involved, considering the unique characteristics of each system. In this way witch species are adapted to provide productions and environmental services.

The forages species adaptation in an ICLS with trees depends mainly of the ability to growth in shaded areas caused by the trees. In shaded conditions forage plants can change its structure and nutritional content. The production and nutritional value is important tools to select forages species for use in shaded areas to use in integrated crop livestock system with trees.

The radiation level that reaches the understory of the ICLS with trees is crucial to the development and the success of the system, as the forages species should be agronomic, environmental and economically viable.

Integrated crop livestock system is an agroecosystem able to conserve natural resources and enhance ecosystem services while maintaining productivity (FAO, 2010). Managing a system for multiple environmental services requires a high

degree of biodiversity in soil organisms, plant communities, and cropping and grazing systems distributed across the landscape.

Environmental benefits of grassland ecosystems can, however, be progressively impaired as intensification of production increases (Lemaire et al., 2014).

The diversification of crop system, as introducing grasslands can provide an improvement in ecological services as reducing the green house gas emission to the atmosphere (Sanullah et al., 2014). Nitrous oxide emissions are among of the most important greenhouse gas emission, contributing 6% to global warming. Agriculture contributes to the increase in atmospheric emissions of  $N_2O$  accounting for 24% of the global annual emissions (IPCCC, 2007). The  $N_2O$  emitted from soils are produced by different soil-related factors, such as moisture, temperature and nitrogen content (Ranucci et al., 2010).

High N input, typical of intensive agricultural systems, may imply further environmental threats, such as ammonia volatilization and nitrate leaching in aquifers and rivers and lead to substantial losses of applied N-fertilizers (Del Grosso et al., 2006). Quantification of  $N_2O$  fluxes emitted from arable soils remains a major challenge. Field measurements of emissions conducted in different soil and agricultural conditions are still scarce and annual estimates are generally assessed from a small number of measurements. They are therefore not necessarily representative of average emissions per year, especially as emissions of nitrogen oxide ( $N_2O$ ) can be very sporadic (Laville et al., 2011).

This thesis is organized into chapters that show, in different ways, as general objective in evaluate the yield, nutritive value and the nitrous oxide soil emission of forages in different integrated systems.

The specific objectives of each chapter are:

Chapter 1: Quantify the productivity and nutritive value of six forage species grown in different management practices.

Chapter 2: Quantify the  $N_2O$  fluxes before and after grassland conversion to a crop rotation.

Chapter 3: Measure the  $N_2O$  soil emission at the same time in grazing and mowed grassland.

## 2. CHAPTER 1

Productivity and nutritive value of six forages grasses grown in integrated crop livestock<sup>1</sup>

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<sup>1</sup> Prepared in accordance with the standards of the Agroforestry System Journal

Productivity and nutritive value of six forages species grown in integrated crop livestock

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## Abstract

The integrated crop livestock systems (ICLS) are alternative to sustainable timber, livestock and crop production. However, for the success of these systems, it is necessary to choose the correct forage and system management given the needs of ICLS. Therefore an experiment was conducted to test several forages species and system management practices : *Urochloa brizantha* cv. Marandu (Ub), *Cynodon spp* hybrid Tifton 85 (Cd), *Hemarthria altissima* cv. Florida (Ha), *Megathirus maximus* cv. Aruana (Mm), *Paspalum notatum* cv. Pensacola (Pn) and *Axonopus catharinesis* (Ac). The experiment was conducted during 2012 in a factorial 2x2x6, with three replicates. Formed by two systems (ICLS with trees and full sun (FS)), two nitrogen rates (0 and 300 kg ha<sup>-1</sup>year<sup>-1</sup>) and the six forages species. The forage dry matter yield (DMY), digestible dry matter yield (DDMY), crude protein yield (CPY), leaves, stem and senescent material percentage were determined. The FS had an increase of 41%, 50% and 35% in DMY, CPY and DDMY, respectively, regardless the specie and nitrogen dose. With the nitrogen application the DMY increased in 33%. The species with higher DMY was Ha (14,0 Mg ha<sup>-1</sup>), although Ha had lower leaves proportion. The species with more leaf blades was Ub (82%). Therefore, species choice, nitrogen fertilizer application and the correct pasture management are strategies that increase forage quality with a potential positive impact on ruminant performance.

**Key words:** pasture, quality, integrated crop livestock system.



## 1. Introduction

The use of integrated crop livestock systems (ICLS) is an efficient alternative for sustainable production (Nair 1993). There are several benefits pointed in this systems, such as for the soil fertility conditions, better utilization of solar radiation (Lin et al. 2001), increased in biodiversity and improved land use (Varella et al. 2009).

The increase of soil fertility in ICLS has been observed in several regions, due the utilization of nutrients by the trees in the soil layers that are beyond the reach of forages roots (Nair 1993). However, it is known that the presence of trees in the system reduces the light available for forages, condition that influences the forage productivity and quality (Barro et al. 2008; Lin et al., 2001; Paciullo et al., 2013).

With the light reduction in the system, the soluble carbohydrates in plants decreases (Belesky 2005). Generally, also occurs the increase of the cell wall content and consequent reductions of the forages digestibility (Lin et al. 2001; Castro et al. 1999). Some studies also reported the increased of lignin content in shaded plants, which contributes for reductions on digestibility (Senanayake 1995). Contradicting the results mentioned above, some studies show a reduction in cell wall content and increased in the digestibility of shaded plants (Kephart and Buxton 1993; Deinum et al. 1996; Paciullo et al. 2007). For C<sub>4</sub> species, in general, the shading effects of shading causes decreases in forage production (Soares et al. 2009; Castro et al., 1999; Burton et al., 1959), and the effects on nutritive value of the forage are not well understood (Jackson and Ash 1998). Lin et al. (2001) found different responses to light reduction in 30 forage species, and this response varies according to the degree of plant tolerance to shade.

The luminosity reduction in ICLS depends on the tree population, thus manipulating this fact in the system is a strategy adopted to modify the biomass production of the other components by the control of intra and interspecific competition (Ribaski 2008). The trees

were planted as alley crop system allows greater light entrance in the system with higher dry matter production (Pofirio- da-Silva et al. 1998).

In this context, Cooper and Wilson (1970) postulated that the canopy efficiency in the conversion of light energy into biomass depends on the individual leaves photosynthetic rates and the patterns of canopy light interception. The accumulation of dry matter in forages is the result of environmental interactions and its effects on physiological processes on the morphological plants characteristics (Da Silva and Pedreira 1997). The pasture management with optimal LAI (95 % light interception) allows maximum leaf accumulation of leaves in relation to the stem, improving forage quality (Trindadet al. 2007; Lin et al., 2001; Wilson et al., 1982), in full sun systems. Although in shade environments, as ICLS with trees, the criteria for cut at 95% of light interception may be alter.

The nitrogen fertilization also plays an important role in plant quality and growth. Nitrogen is directly linked with light capture by the plant and leaf growth (Lemaire et al., 2007), According to Valladares and Niinemts (2008) nitrogen can affect the plant response to shade, altering their growth and development capacity.

In this context a few studies in integrated crop livestock systems had evaluated forage by using light interception as harvest criteria for integrated systems.

Our aim is to characterize the response of forage species in association with trees and, consequently, indicate their potential for use in sustainable ICLS. Therefore, an experiment was carried out in order to quantify the productivity and nutritive value of six forage species grown in full sun and in an association with trees (i.e. an ICLS).

## **2. Material and Methods**

The experiment was conducted at the Agronomic Institute of Paraná (IAPAR), located in Ponta Grossa - PR (25°07'22"S ; 50°03'01"W). The climate is Cfb, according to Koppen's

classification, i.e. a subtropical mesothermal humid. The temperatures of the coldest months range from  $-3^{\circ}\text{C}$  to  $18^{\circ}\text{C}$ , and temperatures in the hottest months are between  $10^{\circ}\text{C}$  to  $34^{\circ}\text{C}$ . The region has no defined dry season, with annual rainfall between 1200-1600 mm. The mean relative humidity is between 70-80 % (IAPAR 2000). The soil is an Oxisoil, medium texture classification according to EMBRAPA (2006).

The design of the experimental area was in a factorial  $2 \times 2 \times 6$ , with three replications. Two systems (full sun and ICLS), six  $C_4$  forage species (*Axonopus catharinensis* (Ac), *Cynodon* spp. Hybrid Tifton 85 (Cs), *Hemarthria altissima* cv. Florida (Ha), *Megathirus maximus* cv. Aruana (Mn), *Paspalum notatum* cv. Pensacola (Pn) and *Urochloa brizantha* cv. Marandu (Ub)), usually used in Brazil and also recommended (Soares et al. 2009) for use in ICLS and two nitrogen rates (0 and  $300 \text{ kg N ha}^{-1} \text{ year}^{-1}$ ).

Nitrogen fertilizer was applied at the beginning of each growing season (October). The correction of soil acidity and fertilizer  $\text{P}_2\text{O}_5$  and  $\text{K}_2\text{O}$  were performed according to soil analysis, in each year.

The *Eucalyptus dunnii* trees were planted in 2007 in double rows, with 3m between trees within rows and 4m between rows, with spaced 20m apart ( $3 \times 4 \times 20\text{m}$ ), fitting to an east – west orientation, following the contour. The initial population was  $267 \text{ trees ha}^{-1}$ . In winter 2011 a thinning management was performed reducing the number of trees to  $155 \text{ trees ha}^{-1}$ . A pruning was done in 2012.

The forages species were planted in January 2010 in plots of  $4.5 \text{ m}^2$  ( $1.5 \times 3 \text{ m}$ ) in full sun (no crop integration) and in the shaded area (ICLS) in plots of  $100 \text{ m}^2$  ( $5 \times 20 \text{ m}$ ). In all plots a cut of standardization were performed at 10 cm height.

The interception of forest canopy (i.e. shading percentage) was measured with the aid of two ceptometers, one placed in full sun and another under the forest canopy. From the

difference of the readings of the two ceptometers, the decrease of light interception in the forest understory was calculated.

The forages light interception was monitored weekly using a ceptometer (AccuPAR LP-80). Three measurements obtained at full sun plots were in three positions and measurements in the ICLS were in five positions (2, 4, 10, 16 and 18 meters from the trees row), with measurements made at ground level and above the sward, to compose the plot mean. The difference between the two measures represented the intercepted radiation by vegetation. The canopy height was measured 2 times/m<sup>2</sup> with the help of "sward stick"(Bartham, 1986).

The cuts to dry matter yield evaluation were performed by cutting whenever the sward reached levels of incident light interception corresponding to 95% (interval criterion). The height at which the cut was made corresponded to 50% reduction of the initial height (intensity criterion). The samples period were from the year of 2012.

To collect the material sample, were carried out one sampling in the full sun plots, and five sub-samples in the plots of the ICLS for the morphological separation (leaf, stem) and determination of dry matter, crude protein and dry matter digestibility.

For determination of crude protein and digestibility of dry matter, the samples were dried at 60 °C under air flow for 48 hours, milled (1 mm) in Willey mill and analyzed by the method of near infrared reflectance (NIRS) (Marten et al. 1985). Analyses were performed with spectrometer Perstorp Analytical, Silver Spring, MD, 5000 model, coupled to a microcomputer equipped with ISI software version 4.1 (Intrasoft International, University Park, PA). The annual yield of digestible dry matter and crude protein yield were calculated based on the production of dry matter, digestible dry matter and crude protein concentration, for each cut.

The results were analyzed by the Shapiro-Wilk test for homogeneity of variances and then performed the residuals variance analysis (ANOVA, lme procedure). Data were transformed when necessary to reach the normality of residues. Transformations were performed using the Box-Cox procedure (package MASS). The average effect of the treatments (species x system x N levels) was compared by Tukey test using the R program for linear models.

### 3. Results

There was a significant system effect, N level and forage species ( $p < 0,001$ ) in all variables evaluated, excepted for percentage of stem (Table 1). The system in full sun obtained 41% higher dry matter yield than the ICLS with trees, independent of forage and nitrogen applied (Figure 1). Forages grown with nitrogen ( $11,3 \pm 33,06 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ) obtained a 33% more dry matter yield than without nitrogen ( $7,4 \pm 29,46 \text{ Mg ha}^{-1} \text{ year}^{-1}$ ).

Table 1. F ratios and ANOVAs of forages dry matter yield (DMY), crude protein yield (CPY), digestible dry matter yield (DDMY), % of leaves (Leaf), % of stems (Stem) and % of senescent material (Senesc.).

	DMY	CPY	DDMY	Leaf	Stem	Senescent Material
System	59,94***	42,59**	58,69***	12,94**	ns	4,22*
Specie	17,93***	12,28***	16,92***	32,65***	37,90***	16,03***
N	53,90**	101,95***	61,93***	12,26**	ns	17,04***
System x Specie	ns	ns	ns	ns	ns	ns
System x N	ns	ns	ns	ns	ns	ns
Specie x N	2,63*	4,49**	3,09**	ns	ns	2,74*
System x Specie x N	ns	ns	ns	ns	ns	ns

\*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ ; n.s., not significant.

The most productive species, without nitrogen fertilizer was Ha ( $11,2 \pm 0,8 \text{ Mg ha}^{-1}$ ) and Ub ( $9,1 \pm 0,8 \text{ Mg ha}^{-1}$ ) (Figure 2), with nitrogen application the most productive specie was Cd ( $13,9 \pm 0,8 \text{ Mg ha}^{-1}$ ). The specie less productive in both nitrogen doses was Pn

(Figure 2). The specie most responsive to nitrogen application was Cd, followed by Mn, with increments in order of 43 and 40%, respectively.

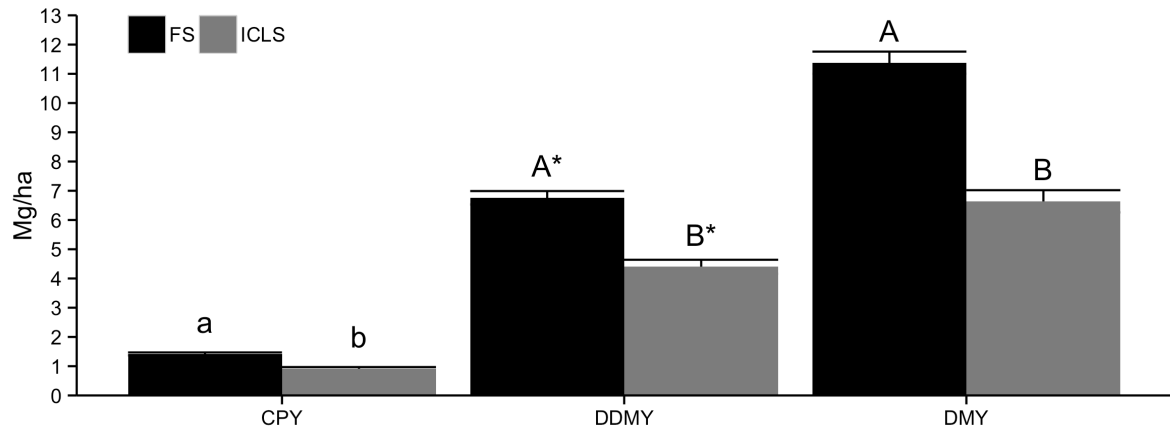


Figure 1. Crude protein yield (CPY, n=2), digestible dry matter yield (DDMY, n=2) and dry matter yield (DMY, n=2), within each light condition (FS= full sun and ICLS = ICLS with trees). The bars show standard error. Means with the same letter are not significantly different

Among the systems studied, the crude protein yield was greater in the full sun system (Figure 1). The addition of 300 kg ha<sup>-1</sup> increased the crude protein yield in 50.4%

Without nitrogen fertilizer the species with the highest protein yield was the Ub 1,0 ± 0,09 Mg ha<sup>-1</sup>, with 300 kg ha<sup>-1</sup> of nitrogen fertilizer the species with most crude protein production was Cd and Ub with 2.0 ± 0,09 and 1.8 ± 0,09 Mg ha<sup>-1</sup>, respectively (Figure 3).

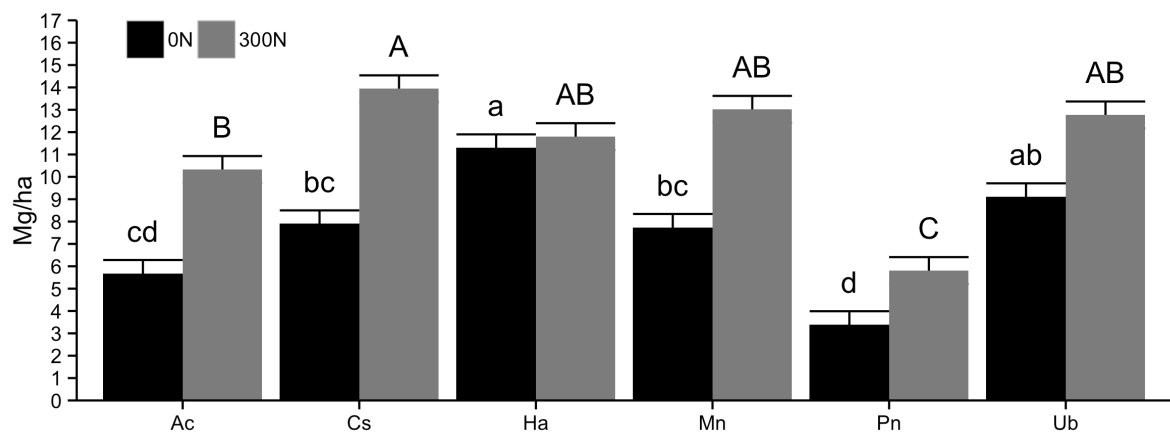


Figure 2. Dry matter yield (DMY) within each nitrogen fertilization condition (N0, no N fertilization, N300, 300 kg.ha<sup>-1</sup>.yr<sup>-1</sup>) and each species (See material and methods for species codes). The bars show standard error. Means with the same letter are not significantly different (N0 = case letters, N300 capital letters).

The digestible dry matter production varied depending on the system and nitrogen dose and specie. On average, the full sun system had 35% more digestible dry matter production, comparing to ICLS with trees. The application of 300 kg ha<sup>-1</sup> increased the digestible dry matter production in 36% (2,4 Mg ha<sup>-1</sup>). The species did not differ from each other for digestible dry matter yield, except the Pn with 3,4 ± 0,4 Mg ha<sup>-1</sup> with the addition of 300 Mg ha<sup>-1</sup> (Figure 4). Without nitrogen application the specie with most digestible dry matte yield was Ha (6,3 ± 0,5 Mg ha<sup>-1</sup>) and the less productive specie was Pn (1,9 ± 0,3 Mg ha<sup>-1</sup>) (Figure 4).

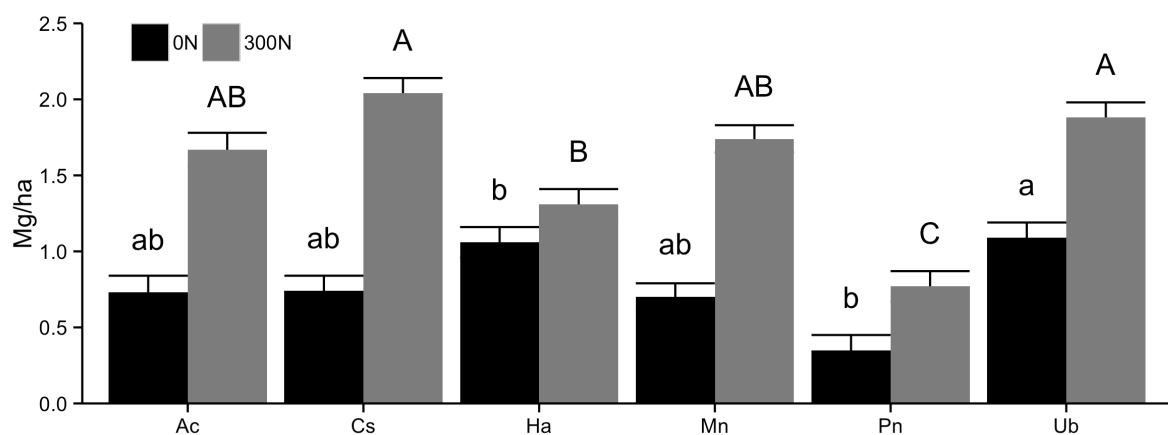


Figure 3. Crude protein yield (CPY) within each nitrogen fertilization condition (N0, no N fertilization, N300, 300 kg.ha<sup>-1</sup>.yr<sup>-1</sup>) and each species (See material and methods for species codes). The bars show standard error. Means with the same letter are not significantly different (N0 = case letters, N300 capital letters).

The analysis of variance shows significant differences between system, species and nitrogen fertilization for the percentage of leaves. For the percentage of stem were only significant differences between species and for percentage of senescent material were significant differences between system, species and nitrogen fertilization (Figure 1).

Nitrogen fertilization resulted in an increase of leaf blades in the order of 8%, and the reduction in the senescent materials proportion (4.3%). The leaves proportion was also altered by ICLS (10%), regardless of the species.

With the application of nitrogen fertilizer the Ub showed the highest proportion of leaves (82%), while Ha obtained only 38%. At the opposite, the proportion of stem ranged

from 11% (Ub) to 53% (Ha) (Figure 5). Regarding the interaction of species with N, just Ub and Pn did not change the proportion of leaf blades in harvested biomass (Figure 5).

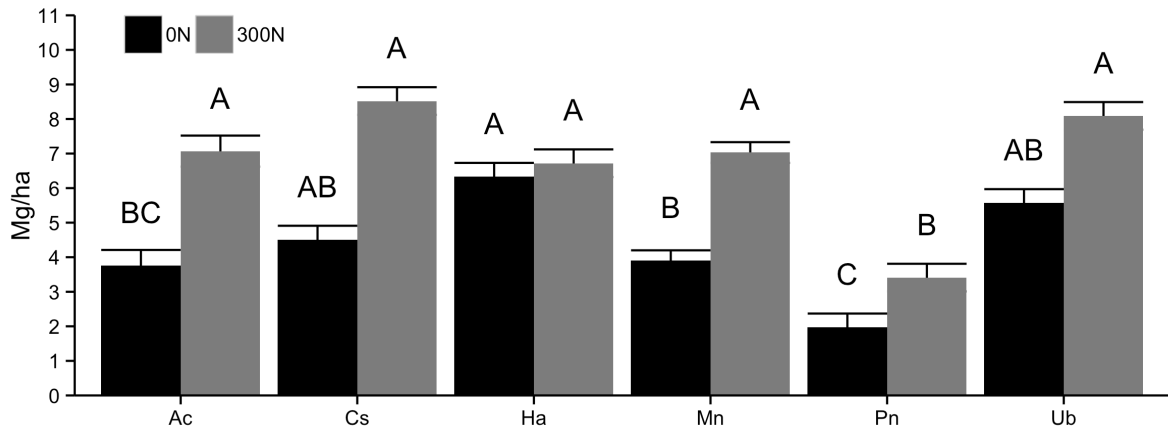


Figure 4. Digestible dry matter yield (DDMY) within each nitrogen fertilization condition (N0, no N fertilization, N300, 300 kg $ha^{-1}yr^{-1}$ ) and each species (See material and methods for species codes). The bars show standard error. Means with the same letter are not significantly different (N0 = case letters, N300 capital letters).

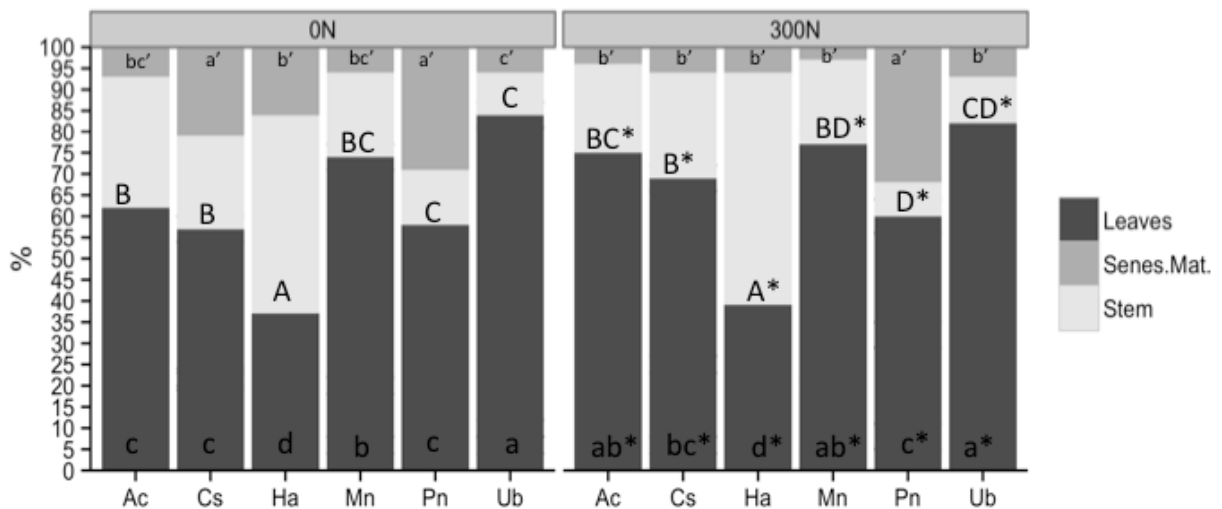


Figure 5. Leaves, Stem and senescent material percentage within each nitrogen dose (N0, no N fertilization, N300, 300 kg $ha^{-1}yr^{-1}$ ) and each species (See material and methods for species codes).

#### 4. Discussion

Gautier et al. (1999) found lower carbon production in ICLS, which affected dry matter production. Here, we also observed a reduction on dry matter yield in ICLS due to the lower incidence of photosynthetic active radiation in the sward in understory.



The highest forage yield obtained with nitrogen fertilization can be attributed to the effects of nitrogen, which promotes an increase in the rates of enzymatic reactions and metabolism of plants as reported by several studies (Alexandrino et al., 2004; Da Cunha et al., 2008; Fagundes et al., 2005; Martuscello et al., 2005). According Colozza et al. (2000), the content of chlorophyll in leaves occurs in plants with higher nitrogen availability, which increases the supply of assimilates influencing morphogenetic and structural characteristics of pastures, as the size and number of tillers, having direct positive impacts on forage production. The increase in forage dry matter yield with nitrogen fertilization was also reported by many studies, for instance, Wilson and Wild (1991), Lopes et al. (2003) and Manzanti et al. (1994).

The dry matter yield and protein yield reduction exhibited by most species in ICLS with trees is in agreement with literature (Shelton et al, 1987; Castro et al, 1999). In fact, in most studies with tropical grasses, there are reports of reduced forage production under shade, due to the marked reduction in photosynthesis of C<sub>4</sub> forage (Deinum et al, 1996; Andrade et al, 2004). The photosynthetic apparatus of C<sub>4</sub> plants have no light saturation even at high radiation intensities, due to the concentration of CO<sub>2</sub> in mesophyll cells mechanism with that C<sub>4</sub> grasses have a large reduction in photosynthesis in low light conditions (Taiz & Zeiger, 2004), as these type of ICLS. Castro et al. (1999) showed 50% reduction in the yield of *U. brizantha* when grown with 60% shading.

When grown in full sun system forages produced greater crude protein yield, due to more protein content (data not showed and also greater dry matter production. According to Corsi (1984), nitrogen fertilization can reduce the percentage of neutral detergent fiber in plants by stimulating the growth of new tissue, which has lower levels of structural carbohydrates in dry matter. Nitrogen fertilization can positively influence the digestibility of forage dry matter, as it stimulates the growth of new tissues that have high protein and low

amounts of structural carbohydrates and lignin in the dry matter (cell wall components). This effect would be more pronounced in tropical forages, which the percentage of cell wall dry matter is inversely correlated to crude protein.

The highest values of leaf:stem ratio for the most of the species, such as *U. brizantha* can be attributed to cutting height, which removed most of the leaf blades in relation to the stem, because leaf blades have higher nutritional value compared to other plant parts (Trindade et al., 2007).

The forage management using the 95% of light intercept by the forage canopy, as frequency criteria, allows high herbage intake rate and animal production, as well as improves the quality of harvested forage (Trindade et al. 2007; Zanini et al. 2012). For the intensity criteria (50% off the height) seems to improve the leaves:stem ratio for all the species studied, less the Ha.

## 5. Conclusions

There was a significant system effect, dose and forage species on dry matter production ( $p < 0.001$ ). The system in full sun obtained a 33 % higher dry matter yield than in ICLS, regardless species and nitrogen level. In general the nitrogen fertilization compensated the yield reduction (-33%) caused by the trees effect with the trees in the ICLS.

The most productive species and the species more adapted to the management (LI=95%) was *Urochloa brizantha*. Also Ub showed better leaves proportion.

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### 3. CHAPTER 2

Grassland management on nitrous gas emissions under temperate climate conditions<sup>2</sup>

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<sup>2</sup> Prepared in accordance with the standards of the Journal Global Change Biology

**Grassland management on nitrous gas emissions under temperate climate conditions**

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## Abstract

Quantifying nitrous oxide emission ( $N_2O$ ) fluxes emitted from croplands remains a major challenge because field measurements in different climates, soil and agricultural systems are still scarce and emissions are usually calculated from a small number of measurements. In this paper we asked: (1) Are the  $N_2O$  soil emission increased after changing grassland to a crop rotation? (2) How long lasting is this effect? (3) What is the influence of fertilization on the  $N_2O$  soil emission under grassland and crop? To answer this questions an experiment in Centre West France was build, with 6 automatic chambers at rate of 16 mean flux measurements per day, in a 3 years crop rotation and permanent mowed grassland at same time from 2010 to 2013. The  $N_2O$  emission were larger for the converted grassland to crop rotation than the permanent mowed grassland (1,52 (1,63)  $ng\ N.m^{-2}.s^{-1}$ , and 1,37 (1,87)  $ng\ N.m^{-2}.s^{-1}$ , respectively (inter quartile range)). After the plowing a  $N_2O$  emission peak occurred for 34 days with flux mean of 5,43 (3,35)  $ng\ N.m^{-2}.s^{-1}$  compared to 1,79 (1,36)  $ng\ N.m^{-2}.s^{-1}$  for the mowed grassland. The drives of  $N_2O$  soil emissions are soil N content and soil water content. The plowing increases the  $N_2O$  soil emission in about 3 times, due to soil disturbance and probably to largest soil microbial activity.

**Key words:**  $N_2O$ , plowing, forage, crops, integrated systems

## 1. Introduction

Agricultural activity strongly impacts greenhouse gas emissions. Worldwide, 23% of the  $CO_2$  emissions and 58% of nitrous oxide ( $N_2O$ ) emissions come from the agricultural sector (Smith et al., 2007, IPCC, 2007); in France and probably other European countries these contributions are even higher with 84% of the  $N_2O$  emissions derived from agriculture (Lopez et al., 2012). Soil is the main source of these emissions. Fluxes of  $CO_2$  and  $N_2O$  from agricultural soils are the result of complex interactions between climate, soil microbial activity, chemical and physical soil properties (Attard et al., 2009). Whereas  $CO_2$  emissions are caused by soil microbial and plant respiration, soil microbial denitrification is the main

source of N<sub>2</sub>O emissions. N<sub>2</sub>O emissions are characterized by very high spatio-temporal variability (Goffmann et al., 2006). These emissions are typically thought to occur under anaerobic conditions, when soil bacteria will reduce nitrogen (N) oxides, such as nitrate (NO<sub>3</sub><sup>-</sup>) to nitric oxide (NO) to N<sub>2</sub>O and last N gas (N<sub>2</sub>) although other pathways including ammonia (NH<sub>4</sub>) oxidation, nitrifier denitrification and chemo-denitrification, which may occur under aerobic conditions, have been reported (Venterea et al., 2012). The net N<sub>2</sub>O and CO<sub>2</sub> flux between the soil and the atmosphere is the result of the balance of production and consumptions of these gases within the soil surface and the contribution for these processes to N<sub>2</sub>O and CO<sub>2</sub> emissions vary with climate, soil conditions and land use (Skiba and Smith, 2000; Laville, 2011). Whereas CO<sub>2</sub> emissions occur continuously depending on climatic conditions, N<sub>2</sub>O emissions are strongly impacted by single events, such as fertilization and rainfall (Butterbach-Bahl et al., 2013). The accurate assessment of these ‘hot moments’ requires continuous emission monitoring. However, up to now many studies in agricultural systems rely on weekly or biweekly emission measurements (Almaraz et al., 2009; Omonode et al., 2011).

The introduction of grasslands into the cropping cycle has been postulated as being beneficial for maintaining soil fertility and agricultural productivity. It seems that this diversification of the regular agricultural management could be an important improvement for the quality of services gained from this system (Sanullah et al, 2014). Temporary grassland management is strongly dependent on N fertilization to maintain crop production as well as pasture productivity and thus susceptible to contribute significantly to N<sub>2</sub>O emissions. In such a system, the most important impact on greenhouse gas emissions are occurring through land-use change by plowing grasslands for installing crop rotations (Sauerbeck 2001; Vellinga et al. 2004). Grassland conversion to arable land impacts soil organic matter stocks and composition (Rumpel and Chabbi, 2004) and also soil nitrification and denitrification

potential (Attard et al., 2011). Its effect on actual greenhouse gas emissions under field conditions has been addressed only in few studies (Almaraz et al., 2009). None of them assessed simultaneously  $N_2O$  and  $CO_2$  emissions through continuous emission monitoring over several years period. However, such datasets are indispensable to validate C and N cycle models (Shaffer and Hansen, 2001).

Considering that a large proportion of annual  $N_2O$  emissions commonly occurs over a timescale of hours or weeks following management or climatic events, such as fertilization, tillage or precipitation (Johnson et al., 2010) we focused in the present study on the impact of grassland turnover and fertilization on  $N_2O$  and  $CO_2$  soil emission under field conditions. We investigated the effect of converting six years permanent grassland to agricultural land on  $N_2O$  soil emission, over a two years period after the conversion. Thanks to the use of a long-term experiment designed to follow agricultural management impacts on biogeochemical cycles, we were able to monitor  $N_2O$  and  $CO_2$  soil emissions of permanent grassland, converted grassland and a crop rotation through continuous measurements on adjacent sites. The aim of our study was to quantify the  $N_2O$  fluxes before and after grassland conversion to a crop rotation. Specifically we asked: (1) Are  $N_2O$  soil emissions increased and linked after changing grassland to a crop rotation? (2) How long lasting is this effect? and (3) What is the influence of fertilization on the  $N_2O$  soil emission under grassland and crop?

## **2. Materials and methods**

### **2.1 Field site**

This study was carried out at Experimental Site of SOERE-ACBB (Agro ecosystem Biogeochemical Cycles and Biodiversity) at Lusignan, Centre-West France (46°25'12,91" N; 0°07'29,35" E). The soil type at the site is Cambisol with a loamy texture (Chabbi et al. 2009). The mean annual temperature is 10.5°C and precipitation is around 600 mm. The

climate is Cfb, a maritime temperate climate. In 2010, 2011 and 2012, when the experiment was carried out, mean annual temperature was 11.1°C with rainfall of 863 mm. The study site is completely flat temporary grassland, which has been under ley cropping systems for more than 50 years.

The experiment started in 2005, when two plots of mowed grasslands were installed next to a continuous agricultural cropland. On 16 March 2011, these grasslands were converted to a maize, wheat, and barley crop rotation.

On 18 April 2011 the converted grassland plots were sown with maize (*Zea mays* L.) and fertilized with 36 kg N ha<sup>-1</sup>. (Solufix). The maize was harvested on 29 September 2011. Thereafter, wheat was sown on 15 November and harvested on 24 July 2012 and fertilized with ammonium nitrate on 2 April and 22 May 2012. Wheat was harvested on 24 July and barley (*Hordeum vulgare*), was sown on 23 October. All major field management operations are summarized in Table 1.

Water fill pore space (WFPS), air temperature and rainfall were recorded throughout the experiment.

The treatments were a mowed grassland (permanent 6 years mowed grassland) and a grass crop rotation (6 years mowed grassland converted in a crop rotation of maize, wheat and barley), using the number of chambers as repetition (6 chambers).

Table 1. Main Crop management operations in the field, 2011 and 2012, for the grass-crop rotation plot.

Date	Culture	Management/Land Use	Quantities
16/03/11	Grass	Plowing	36 kg N.ha <sup>-1</sup> (Solufix)
18/04/11	Maize	Maize sowing	
02/05/11	Maize	N application	
27/09/11	Maize	Maize harvesting	
15/11/11	Wheat	Wheat sowing	
02/04/12	Wheat	N application	80 kg N.ha <sup>-1</sup> (Ammonitrate)
22/05/12	Wheat	N application	40 kg N.ha <sup>-1</sup> (Ammonitrate)
24/07/12	Wheat	Wheat harvesting	
23/10/12	Barley	Barley sowing	

## 2.2 Flux measurements

Flux measurements were carried out on a six year mowed grassland and a converted plot, where six-year mowed grassland was followed by plowing and crop rotation of maize, wheat and barley. N<sub>2</sub>O and CO<sub>2</sub> were measured continuously before the grassland destruction (grass period), during the plowing (plow period) and during the crop rotation (crop period), since 11 October 2010 to 23 October 2012 and compared with the mowed grass for the same period of time. During plowing the chambers were removed for 3 days.

Nitrous oxide and carbon dioxide flux were measured simultaneously from November 2010 to November 2012 using 6 automatic chambers, which are described in detail by Laville et al. (2011). Each chamber occupied a surface of 0,49 m<sup>2</sup> (0,7 x 0,7 m<sup>2</sup>), with the volume of 0,098 m<sup>3</sup>. Each chamber was sampled in 10 seconds intervals, for 15 minutes, through the analyzers, and the outflow of the N<sub>2</sub>O and CO<sub>2</sub> analyzers was fed back to the chamber.

The N<sub>2</sub>O concentrations were measured by infrared absorption spectrometry (Thermo-Environmental Instruments Inc. USA; model 46 C).

For N<sub>2</sub>O fluxes were calculated from the variations over time in the slopes of the gas concentrations using the following equations:

$$F = \frac{SHMP}{RT} \quad (1)$$

Where F is the flux (in ng Nm<sup>-2</sup>s<sup>-1</sup> for N<sub>2</sub>O), S is the slope of concentration variation (dC) over time variation (dT), H is chamber height, in meters, M is the gas molar mass in gram per mol, P is the atmospheric pressure in Pa, R is the ideal gas constant in J K<sup>-1</sup> mol<sup>-1</sup> and T is the temperature in Kelvin.

For calculation of cumulative period emissions, gaps originating from instrumental failure were filled by “look up table” methodology (Mishurov and Kiely, 2011; Falge et al, 2001a; Falge et al, 2001b). Were gap filled 209 days of measurements over the mowed grass followed the crop rotations (29% of total data). The longest periods of missing data were 24/09/2011 to 21/11/11 and 21/3/2012 to 20/06/2012. For the mowed grass plot were gap filled 262 days (36% of data), the long periods of gap filling were 4/01/2011 to 12/5/2011, 23/06/2012 to 13/07/2012 and 9/10/12 to 12/11/2012.

The emission factor for N<sub>2</sub>O were estimated using:

$$EF = \frac{Ne - Nw}{Na} 100 \quad (2)$$

Where EF (%) is the emission factor, Ne is the nitrogen emission, Nw is the bare soil nitrogen emission (background emissions) and Na is the inorganic or organic nitrogen applied. For calculate the emission factor of the plowing period were taken account the pasture green leaf N content (Na).

### 2.3 Statistics analysis

The normality of the distribution of the data was analyzed using the Komorov Smirnov test. Where the standards assumptions of normality were violated, the Kruscal Wallis

test was used to compare the daily flux averages. Differences were considered significant at the P less than 0,05 level. Linear regression analysis and Pearson Correlation were used to identify significant positive or negative relations between trace gas fluxes and environmental drivers. All the calculations, statistical analysis and graphical outputs were determined using software R.

### 3. Results

#### 3.1 Meteorology

The meteorological conditions are shown in figure 1. The rainfall between November 2010 and November 2011 was 616 mm. In the second year between November 2011 and November 2012 higher rainfall of 793 mm was recorded. The summer months received more rain than the winter months. In 2011, we recorded three months of drought, between February and May (Figure 2).

The soil water content (SWC) exhibited large intra-annual variation following the temperature pattern. The SWC during the summer of 2011 was low, due to the drought in may 2011. The soil water content average  $18 (\pm 7,4)\%$  for the two years and the temperature average was  $12 (\pm 6,3) ^\circ\text{C}$  (Figure 1).

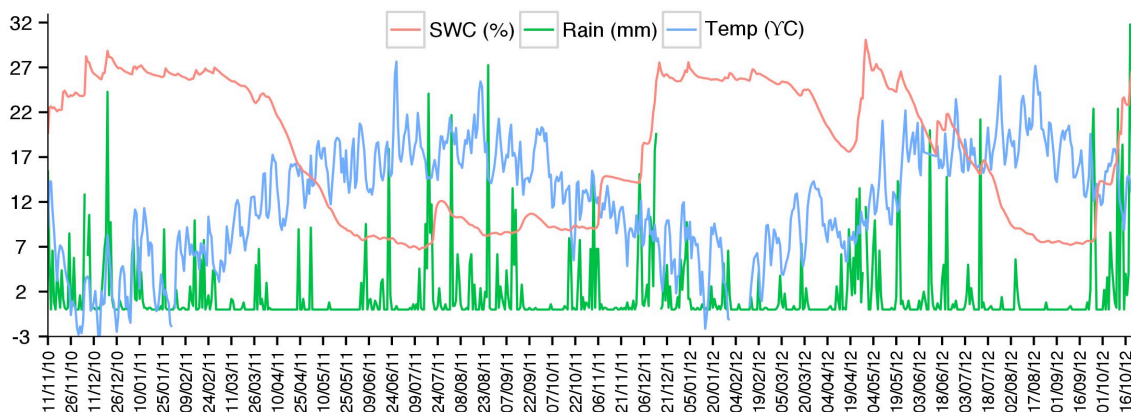


Figure 1 . Mean daily meteorological data. SWC (soil water content), Rain (rainfall) and Temp (Temperature).

### 3.1 Extractable soil nitrogen content

For the crop grassland rotation, the  $\text{NH}_4$  concentration was  $2,97 \text{ mg kg}^{-1}$  in winter 2010. After the winter period, the amount of extractable  $\text{NH}_4$  increased to  $14,45 \text{ mg kg}^{-1}$  (Figure 3), due to greater SWC. After the plowing the ammonium concentration decreased to similar levels than before.

Soil  $\text{NO}_3$  concentrations (Figure 3) showed similar concentrations as  $\text{NH}_4$  until grassland conversion. After the plowing, nitrate concentrations continuously increased to the level of  $21,55 \text{ mg.kg}^{-1}$ , on the first 20 cm of soil.

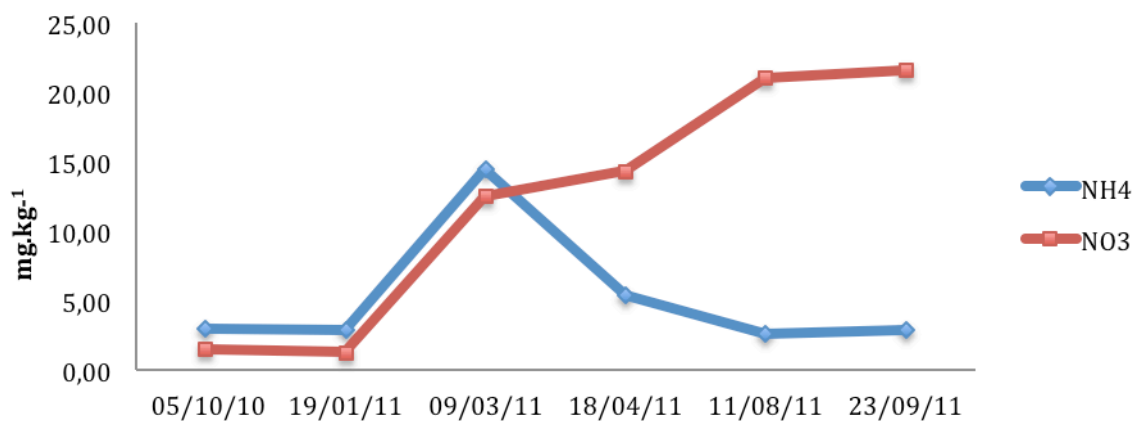


Figure 2. Soil ammonium and nitrate content under grassland crop rotation.

For the mowed grassland the variation in soil nitrate and ammonium content were high (Fig. 4), but the values in the soil under this treatment were always lower as compared to cropland (Fig. 3). Values of soil ammonium were higher in summer ( $8,83 \text{ mg kg}^{-1}$ ) than the winter period ( $2,60 \text{ mg kg}^{-1}$ ).



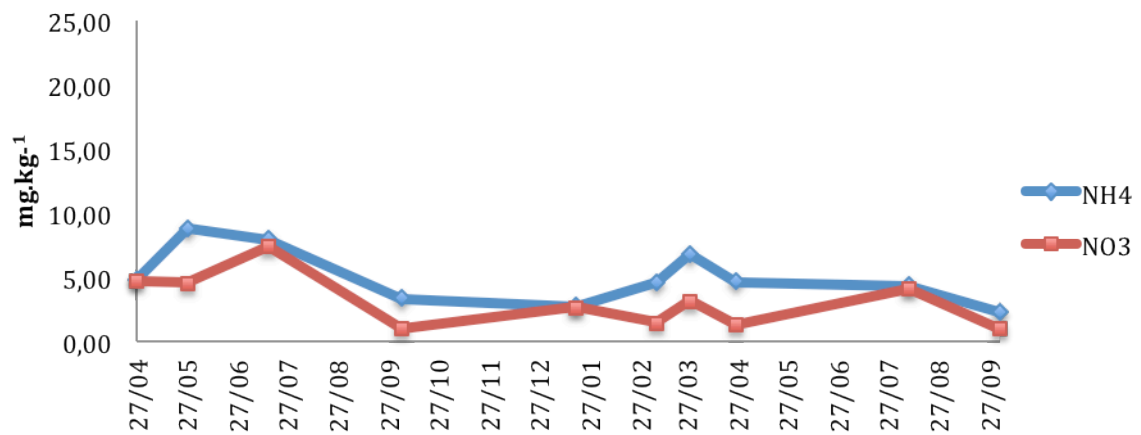


Figure 3. Soil ammonium and nitrate content under mowed grassland.

### 3.2 Nitrous oxide flux spatial variability

Nitrous oxide emissions presented considerable spatial variability in both grass crop rotation and continued mowed grass (Fig. 5). The variability of N<sub>2</sub>O flux measurements made at specific site using 6 chambers was quantified via the coefficient of variation (CV). The CV of flux estimates was 50-395% depending on time of the year and soil management. The spatial variation is similar in both treatments.

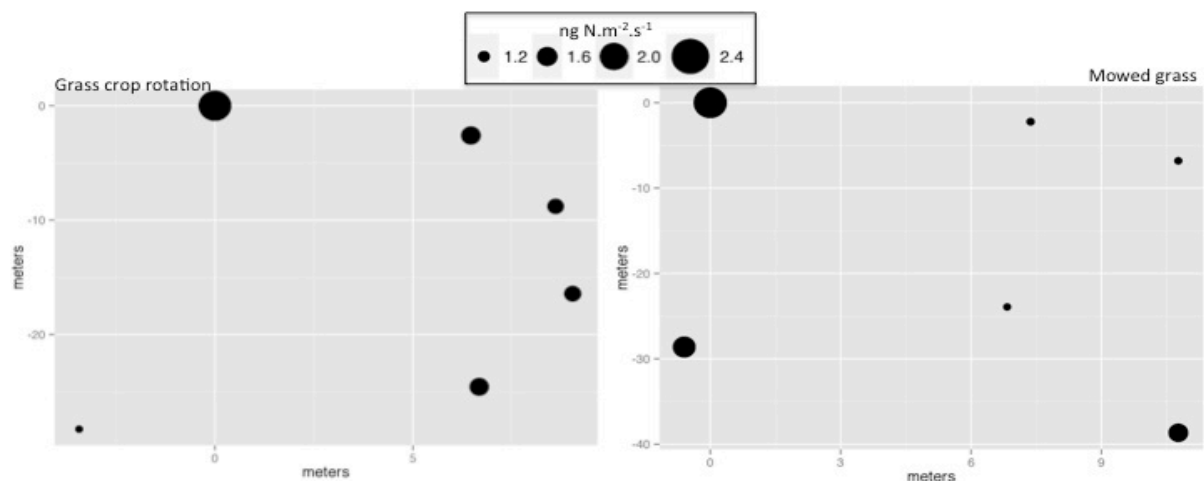


Figure 4. Spatial variation emission of N<sub>2</sub>O under a grass crop rotation and mowed grassland, during the 2010.

### 3.3 Nitrous oxide soil emissions

Variations in N<sub>2</sub>O fluxes rates for both treatments throughout the study period are shown in Fig.6 N<sub>2</sub>O emissions were larger for the converted grassland to crop rotation than the mowed grassland (Fig.6). Mean fluxes over the 2 years measuring period were (inter quartile range) 1,37 (1,87) ng N.m<sup>-2</sup>.s<sup>-1</sup>, from the mowed grass and 1,52 (1,63) ng N.m<sup>-2</sup>.s<sup>-1</sup> from grass crop rotation.

For the grassland converted to crop rotation we observed large N<sub>2</sub>O emissions after plowing, in Mars 2011. The maximum N<sub>2</sub>O emission were 12,22 ng N.m<sup>-2</sup>.s<sup>-1</sup>, on twentieth Mars 2011, and the minimum N<sub>2</sub>O flux was -10,23 ng N.m<sup>-2</sup>.s<sup>-1</sup> before the plowing event on 5/10/2012. The N<sub>2</sub>O emission peaks were largest after plowing and high rainfall events, as in Mars 2011, January, April and September 2012 (Fig. 1 and 4a).

The mowed grassland presented N<sub>2</sub>O emission peaks in July 2011, February and November 2012, with the maximum of 16,24 ng N.m<sup>-2</sup>.s<sup>-1</sup>, in 26/7/2011 and the minimum of -9,15 ngN.m<sup>-2</sup>.s<sup>-1</sup>, in 27/12/2010. The highest N<sub>2</sub>O emission period coincided with rain and nitrogen fertilization after a long period of drought (Fig. 2 and 4b).

The N<sub>2</sub>O fluxes rates, after the plough, showed clear increase 34 days after the grassland destruction. The ploughed treatment showed 3 times more emission than the continued mowed grass, with a daily N<sub>2</sub>O flux mean of 5,43 (3,35) ng N.m<sup>-2</sup>.s<sup>-1</sup> compared to 1,79 (1,36) ng N.m<sup>-2</sup>.s<sup>-1</sup> for the mowed grassland (Fig 7).

The soil N<sub>2</sub>O emissions under maize and wheat did not differ statistically of the N<sub>2</sub>O emission under the mowed grassland, with an emission average of 1,30 (1,13) ng N.m<sup>-2</sup>.s<sup>-1</sup>.

The N<sub>2</sub>O flux peaks between the ploughed grass and the continued mowed grass are different, suggesting different N<sub>2</sub>O emissions drivers for the two types of management.

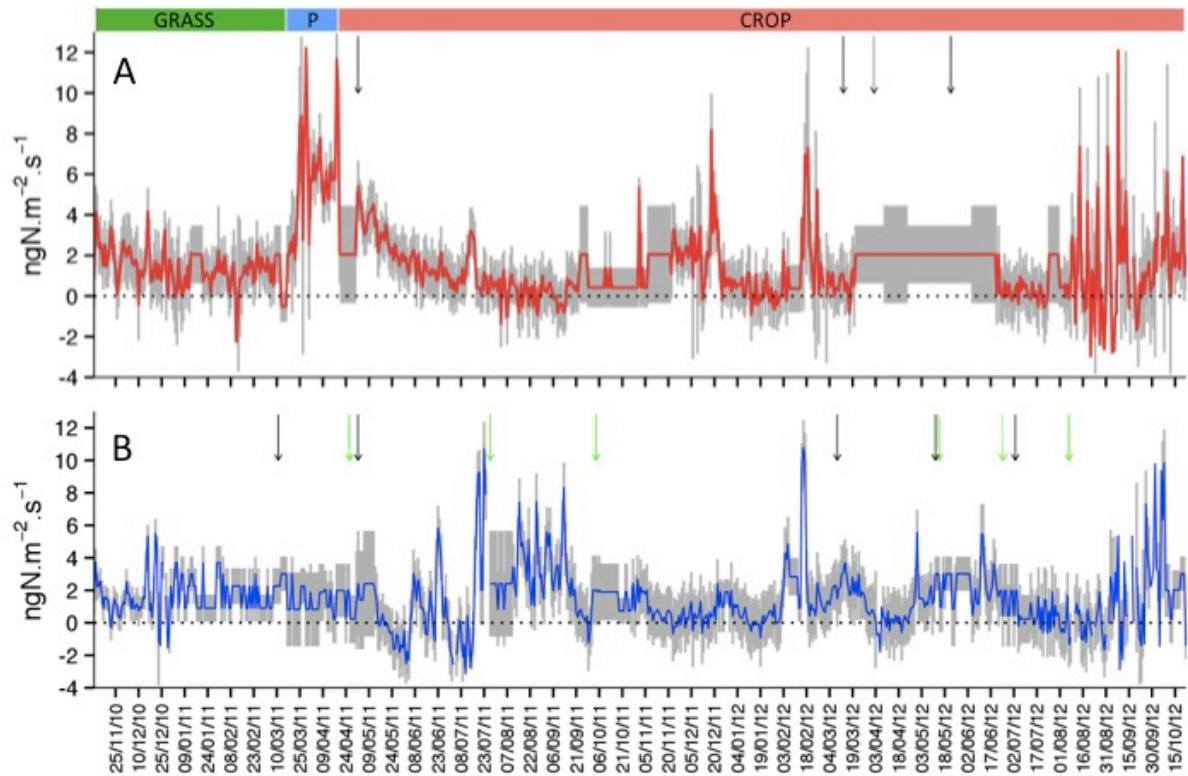


Figure 5. Daily average N<sub>2</sub>O flux of a crop-grassland rotation (A) and pure grassland (B). Black arrows are nitrogen fertilizer application and green arrows are cuts.

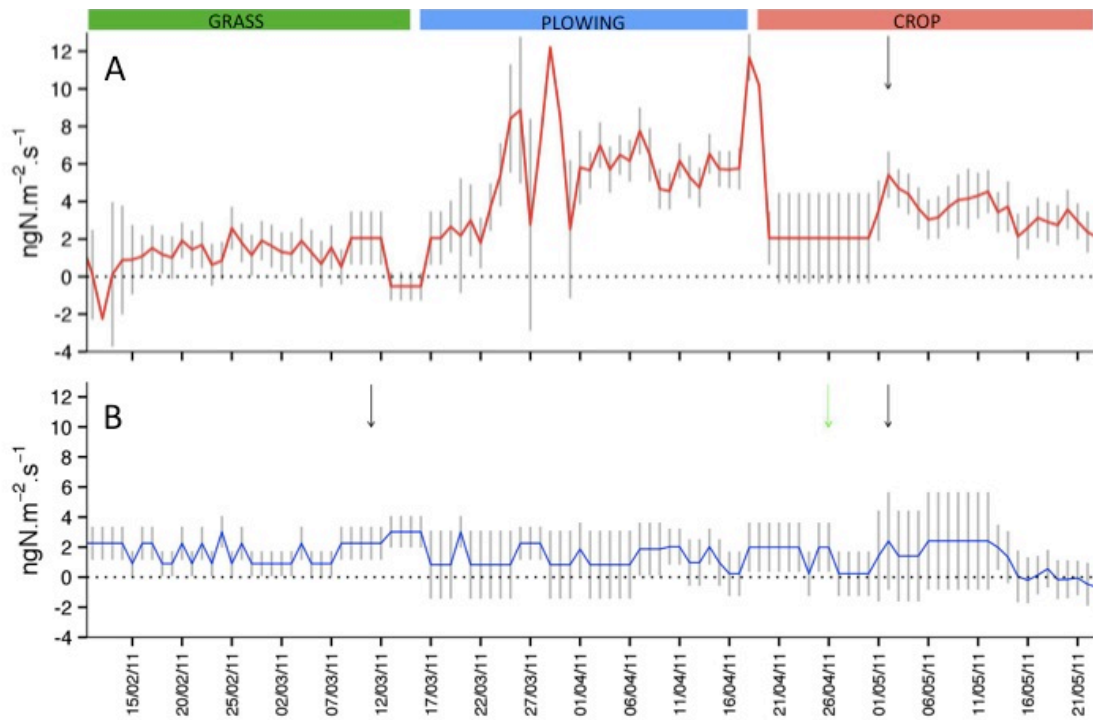


Figure 6. Daily average N<sub>2</sub>O flux 34 day before and after the plowing of a crop-grassland rotation (A) in comparison with pure grassland (B). Black arrows are nitrogen fertilizer application and green arrows are cuts.

The cumulated  $\text{N}_2\text{O}$  emission were higher for converted grassland than for the mowed grassland, 936,63 and 834,48  $\text{g N ha}^{-1} \text{d}^{-1}$ , respectively (Fig. 8). The greatest difference during the 34 days after the grassland plowing, with 159,59  $\text{g N ha}^{-1} \text{d}^{-1}$  and 45  $\text{g N ha}^{-1} \text{d}^{-1}$  for grass crop rotation and mowed grassland, respectively. Thereafter, grassland seemed to emit less  $\text{N}_2\text{O}$  than grass crop rotation.

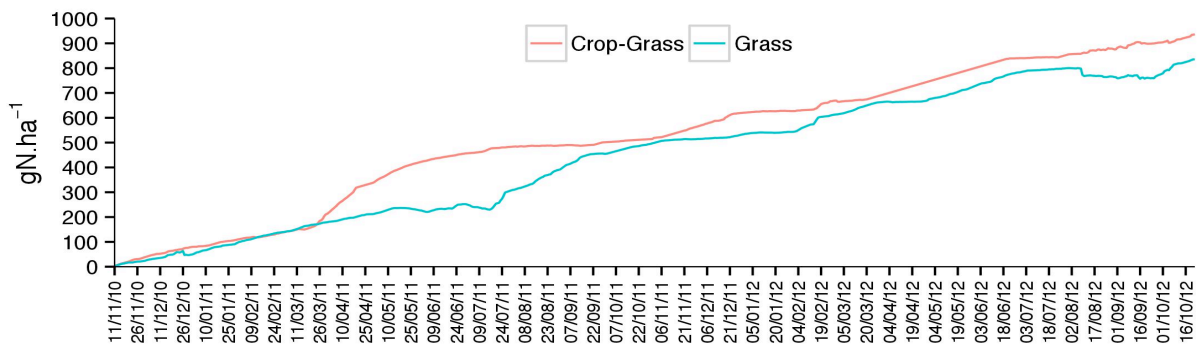


Figure 7. Cumulated  $\text{N}_2\text{O}$  emission for crop grassland rotation and grassland for two years.

### 3.4 $\text{N}_2\text{O}$ emission factor

The  $\text{N}_2\text{O}$  emission factor ranged between, 0.13 and 0.27 %. The grassland crop rotation emission factor was 0,24%, while for the permanent grass the emission factor was 0,19%. During the crop period the emission factor for the grass crop rotation was 0,31%. Indicating that the crop period was the major drive for the increase in the  $\text{n}_2\text{o}$  soil emission. (Table 1).

The period of most  $\text{N}_2\text{O}$  emission were 34 days after the plowing, but emission factor during this period were low, 0.029%, taking account a increment of 471  $\text{kg ha}^{-1}$  of nitrogen from grassland green leaves.

Table 2 . N<sub>2</sub>O soil emission factor under mowed grassland and grass crop rotations, during maize and wheat.

Grass Crop Rotation				
Culture	Date	Fertilization (kg N ha <sup>-1</sup> )	N <sub>2</sub> O emission (kg N <sub>2</sub> O-N/ha)	EF (%)
Maize	19/04/11 - 27/09/11	36	0,19	0,26
Wheat	27/11/11 - 24/07/12	160	0,28	0,09
<b>Total</b>	<b>11/11/10 - 22/10/12</b>	<b>196</b>	<b>0,94</b>	<b>0,27</b>
Mowed Grassland				
Culture	Date	Fertilization (kg N ha <sup>-1</sup> )	N <sub>2</sub> O emission (kg N <sub>2</sub> O-N/ha)	EF (%)
Grass	10/02/11 - 15/03/11	60	0,05	0,05
Grass	16/03/11 -18/04/11	60	0,04	0,03
Grass	27/11/11 - 24/07/12	210	0,28	0,07
<b>Total</b>	<b>11/11/10 - 22/10/12</b>	<b>330</b>	<b>0,83</b>	<b>0,13</b>

### 3.5 N<sub>2</sub>O emissions vs environmental variables

There was a correlation between temperature and N<sub>2</sub>O flux, indicating higher N<sub>2</sub>O emission rates at low temperatures. The soil water contents influenced the N<sub>2</sub>O emission more than temperature (Table 3). The largest N<sub>2</sub>O fluxes were recorded after a big rainfall and high soil water content.

Table 3. N<sub>2</sub>O and CO<sub>2</sub> soil emissions and environmental conditions Pearson's correlations, under a grass crop rotation and mowed grass.

	Rain	SWC*	T (°C)	CO <sub>2</sub>	N <sub>2</sub> O
Flux	Grass- Crop Rotation				
CO <sub>2</sub>	0,0412	0,283**	0,434**		0,1694**
N <sub>2</sub> O	0,0044	0,152**	-0,029*	0,1694**	
	Mowed Grass				
CO <sub>2</sub>	0,0071	-0,044	0,339**		0,394**
N <sub>2</sub> O	0,0924*	0,090*	-0,042*	0,394**	

\*SWC = Soil Water Content

#### 4. Discussion

Grassland plowing during land use change lead to significant N<sub>2</sub>O and CO<sub>2</sub> emissions compared to continuously mowed grassland.

The soil N<sub>2</sub>O flux under continuously mowed grassland was lower compared to the cropland, most probably due to lower nitrogen fertilizer application, temperatures and water soil content (Laville et al. 2011). Microbial activity is affected by the environmental conditions, especially temperature. The larger emission period were different for grass crop rotation and mowed grass, indicated that different management systems cause different emissions. The mowed grass received more nitrogen fertilization, but emitted less N<sub>2</sub>O that the grass crop rotation, probably due to less soil disturbance. (Velinga et al., 2004; Attard et al., 2011).

The N<sub>2</sub>O emission peaks for the grass crop rotation coincided with a large rainfall and soil management (plowing and seeding). The plowing contributed, in 34 days, for 16% of the N<sub>2</sub>O emission of the entire emission of the study period. This N<sub>2</sub>O

emission peak, 34 days after the grass plowing, added to the soil large amounts of plant material leading to an increasing of a microbial and fungal growth (Sanuallah et al. 2011). Li et al (2013) demonstrated the increment of soil respiration with the addition of plant material, in controlled conditions. The plant residual mineralization, probably lead to a low C:N ratio and high  $\text{NH}_4$  soil content, enhancing the nitrifiers microbial activity, leading to greater  $\text{N}_2\text{O}$  emissions, just for a period of high microbial activity.

It was likely that a significant portion of  $\text{NH}_4$  was still oxidized to  $\text{NO}_3$  by soil nitrifiers following the addition of plant materials and soil microbes assimilated  $\text{NO}_3^-$  rather than  $\text{NH}_4^+$  for growth. This possibility seems to be well supported by the high  $\text{NO}_3$  soil content, after plowing. Approximately 30 days, after the soil disturbance, as  $\text{O}_2$  became more limiting, less  $\text{N}_2\text{O}$  is produced as the byproduct from nitrification and/or as the intermediate product from denitrification (Myrold et al., 2007; Miller et al., 2008).

The Pearson correlation analysis showed positive relations between soil water content and  $\text{N}_2\text{O}$  soil emission, (Table 3). These results are consistent with those of Wu et al. (2013) who reported that drought turns soil from an  $\text{N}_2\text{O}$  source to zero emission or sink of  $\text{N}_2\text{O}$ , which indicated that  $\text{N}_2\text{O}$  emission tended to increase with an increase in soil moisture content.

As soil water content increases, denitrification became the dominant process for  $\text{N}_2\text{O}$  emission, due to restriction of  $\text{O}_2$  diffusion into the soil (Wolf & Russow 2000; Jäger et al., 2011). The high soil  $\text{NO}_3$  concentration and soil water content favored the nitrification activity (Zhu et al., 2013).

## **5. Conclusion**

The plowing increases soil  $\text{N}_2\text{O}$  soil emissions, after grassland destruction the  $\text{N}_2\text{O}$  flux increased 3 times in a 34 period long, 34 days after the plowing the fluxes

remains low comparing to other flux studies. Among the factors controlling the large nitrogen oxide emission at Lusignan site, soil N mineral content and soil water content was the major factor. Crop Grasslands rotations emit more N<sub>2</sub>O soil than pure grasslands, due to more soil disturbance.

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#### 4. CHAPTER 3

Nitrous gas emissions on mowed and grazed pasture under temperate climate conditions<sup>3</sup>

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<sup>3</sup>Prepared in accordance with the standards of the Biogeosciences Journal

Nitrous gas emissions on mowed and grazed pasture under temperate climate conditions

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## Abstract

The N<sub>2</sub>O emitted from soils is considered as one of the major contributors to greenhouse gas emissions. The soil properties and processes including soil temperature, soil moisture and N soil content among others determine the ecological process that influences N<sub>2</sub>O emission. Pasture management practices such as grazing and mowing may influence the nutrients balance of the system, subsequently affecting the grassland N cycle, including soil N<sub>2</sub>O emission. In order to understand N<sub>2</sub>O soil emission and compare those practices, an experiment was carried out in West of France. Mean N<sub>2</sub>O emission rates varied from a range of -5,63 to 32,60 ngNm<sup>-2</sup>s<sup>-1</sup>, with means of 2,96 (±0,21) and 2,17 (±0,15) ngNm<sup>-2</sup>s<sup>-1</sup> for the mowed pasture and grazed pasture, respectively. The cumulated N<sub>2</sub>O emission were higher in the mowed grassland (p<0,001) - 1596,25 gNm<sup>-2</sup> – compared to 1173,09 gNm<sup>-2</sup> in the grazed pasture. Soil moisture and soil N content were the main N<sub>2</sub>O soil emission drivers. Grazed pasture releases less nitrous oxide to atmosphere than mowed pastures, although grazed pasture exhibits more N<sub>2</sub>O emissions per kg of nitrogen applied than mowing.

## 1. Introduction

Pasture management practices may influence the nutrients balance of the system. Pasture mowing determines the removal of plant parts that may have negative effect on plant growth and carbon allocation (Ferraro and Oesterheld, 2002). Root exudations and the rizosphere organisms that affect the nitrogen released from the plants roots mediate this process (Hamilton et al., 2008). Removing plants parts by mowing inevitably provokes the adjustment of root system size and thus causes plant tissue death leading to decomposition and nitrogen mineralization, nitrification and denitrification. Mowing also reduces the input of above ground litter into the soil (Valko et al., 2012), and consequently decreases the amount of coarse organic matter (Mikola et al., 2009) and related gas emissions from soil, including nitrous oxide emissions (N<sub>2</sub>O).

The N<sub>2</sub>O emitted from soils is considered as one of the major contributors to the rise of greenhouse gas emissions (Laville, et al. 2011). The ecological process that influences the N<sub>2</sub>O emission from soils is determined by the soil properties and

processes including soil temperature, soil moisture and substrate viability (Zhang et al., 2012).

The grazing and mowing alter soil properties in grasslands, subsequently affecting the grassland N cycle, including soil N<sub>2</sub>O emission. Wolf et al. (2010) found that grazing decreased soil N<sub>2</sub>O.

Sorensen et al. (2008) reported that mowing altered N cycling by decreasing soil N mineralization. Random deposition of urine and faeces in grazed pastures was also reported to increase N<sub>2</sub>O emission (Zhang et al., 2012). Therefore, there is a need to quantify the changes in N<sub>2</sub>O emission caused by mowing and grazing to fully understand the regional budget of trace gases.

In order to accomplish this requirement, an experiment setup was carried out in West of France to measure the N<sub>2</sub>O soil emission on grazed and mowed grassland. We hypothesize that grazing will increase N<sub>2</sub>O emission due to the removal of a part of plants above the soil surface more intensively than mowing, also grazing can reduce root production reducing soil carbon and C/N ratios, which can result in continuous decreases in the availability of substrate and nutrient for N<sub>2</sub>O production.

## 2. Material and methods

### 2.1 Study Site

The experiment was carried out at the Experimental Site of SOERE-ACBB (Agro ecosystem Biogeochemical Cycles and Biodiversity) at Lusignan, Centre-West France (46°25'12,91" N; 0°07'29,35" E). The site soil is a Cambisol with loamy texture (Chabbi et al., 2009).

The study site is a completely flat permanent grassland which has been cropping for more than 50 years. The mean annual temperature is 10.5°C and precipitation is around 600 mm. During the years of 2010, 2011 and 2012, the mean annual temperature was 11.1°C with the rainfall was 863 mm.

The SOERE-ACBB was established in 2005, when two plots were installed (70 x 50m), one pasture grazed and another mowed. The grasslands consisted of a mixture of three grass species (*Dactylis glomerata* L., *Lolium perenne* and *Festuca arundinaca*).

In the mowing system, grass biomass is harvested as hay and stores as off site animal feed for the off seasons particularly summer drought and winter. However, for better economical return, any off site animal excreta fraction typically is not returned in the mown grassland system and is instead applied to different cropping systems in the region.

In the grazing system, dairy cows grazed the plot frequently and all the excreta was directly added on site along with additional nitrogen fertilizer (Table 1). In order to maintain the two systems (mowing and grazing) in similar plant nitrogen nutrition status, nitrogen fertilizer applications were adjusted taking into account plant nitrogen nutrition status (Lemaire and Meynard, 1997).

Table 1. Number mowing events, amount of harvested hay, number of grazing events, annual animal stocking (mature milk cows with average body weight of 625 kg) rate, number of applications and amount of nitrogen fertilizer applied during the study period 2008-2010 in the mowing and grazing management.

mowing system				
Year	No. Mowing event (number year <sup>-1</sup> )	Amount of harvested hay (g C m <sup>-2</sup> year <sup>-1</sup> )	No. fertilization (number year <sup>-1</sup> )	Amount of fertilizer (kg N ha <sup>-1</sup> year <sup>-1</sup> )
2008	3	483	4	330
2009	3	377	3	230
2010	3	212	3	210
grazing system				
Year	No. Grazing events (number year <sup>-1</sup> )	SR (head ha <sup>-1</sup> year <sup>-1</sup> )	No. fertilization (number year <sup>-1</sup> )	Amount of fertilizer (kg N ha <sup>-1</sup> year <sup>-1</sup> )
2008	9	1,6	4	170
2009	7	1,58	2	110
2010	8	1,09	2	110

## 2.2 Flux Measurements

The N<sub>2</sub>O soil emissions were measured from 9/23/2008 to 3/15/2010 with six automatic chambers (0.49m<sup>2</sup>, 0.30 m high, depth of insertion 10 cm). During the closure period (15 min), the air was circulated through the chambers and passed sequentially through a N<sub>2</sub>O analyser (Thermo 46C: Thermo Electron, Saint Aubon, France) with sensitivity levels of approximately 2 ppb. This system allows measuring fluxes 16 times per chamber day<sup>-1</sup>. The N<sub>2</sub>O flux was calculated by fitting the kinetics of gas concentration to a linear or exponential model (Laville et al., 2011).

The emission factor for N<sub>2</sub>O were estimated using:

$$EF = \frac{Ne - Nw}{Na} 100 \quad (2)$$

Where EF (%) is the emission factor, Ne is the nitrogen emission, Nw is the bare soil nitrogen emission and Na is the inorganic or organic nitrogen applied. In order to calculate the emission factor of bare soil (background emission) we used the accumulated N<sub>2</sub>O soil emission from 539 daily averages from 2009 and 2010.

### 2.3 Statistical analysis

The normality of data distribution was analyzed using the Komorov Smirnov test. Where the standards assumptions of normality were not confirmed, the Kruskal Wallis test was used. Differences were considered significant at P less than 0.05 level. Linear regression analysis and Pearson Correlation were used to identify positive or negative relations between trace gas fluxes and environmental drivers. All calculations, statistical analysis and graphical outputs were performed using R software.

## 3. Results

The climate condition during the evaluation period is shown in Figure 1. The precipitation between September 2008 and March 2010 was 1054 mm. The maximum precipitation was registered in November and December 2009 and was 263 mm.

The soil water content (SWC) presented variation along the year, and was higher in autumn and lower during summer for the years evaluated. The maximum SWC was registered in January 2009 (34.04%), the average SWC during the experimental period was 23%, data show in Figure 1. The mean temperature was 10°C, with a minimum of -7°C and maximum of 25°C.



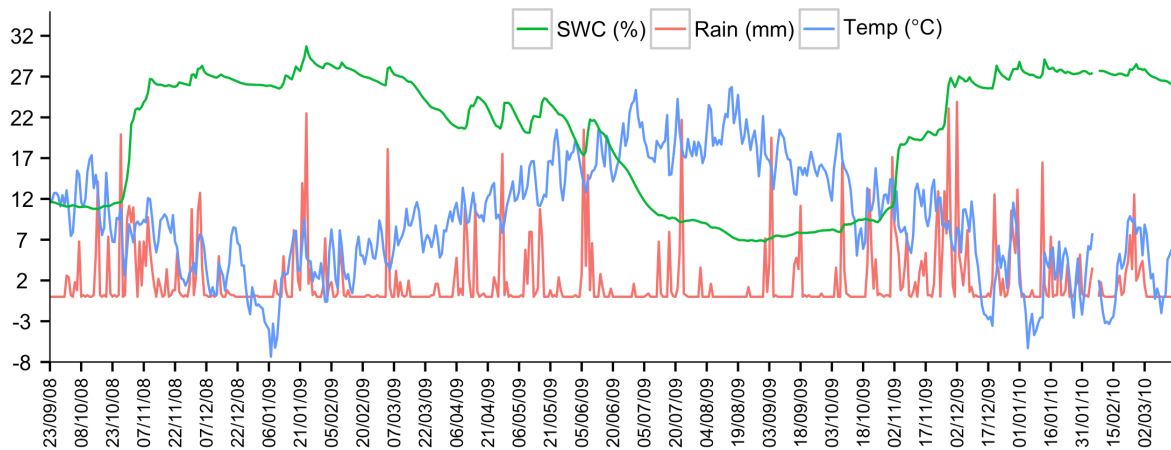


Figure 1. Mean daily meteorological data.

Mean  $\text{N}_2\text{O}$  emission rates from the both management system varied in a range of  $-5.63$  to  $32.60 \text{ ngNm}^{-2}\text{s}^{-1}$ , with means of  $2.96 (\pm 0.21)$  and  $2.17 (\pm 0.15) \text{ ngNm}^{-2}\text{s}^{-1}$  for the mowed pasture and grazed pasture, respectively. The variation of  $\text{N}_2\text{O}$  fluxes was relatively low during autumn and winter, compared to spring and summer. The higher variation in  $\text{N}_2\text{O}$  emission rate resulted in high variation of cumulative  $\text{N}_2\text{O}$  flux. The cumulative flux indicates that grasslands would mainly function as a  $\text{N}_2\text{O}$  source (positive value and slope), but it has also acted as  $\text{N}_2\text{O}$  sink (negative flux values and negative slope). Grasslands acted as sink in July and September for the grazed pasture (Figure 2).

The mowed grassland  $\text{N}_2\text{O}$  soil emission peak was in July 2009, with the maximum of  $26.45 \text{ ngNm}^{-2}\text{s}^{-1}$ , and minimum  $\text{N}_2\text{O}$  soil emission was in August 2009, with  $-2.45 \text{ ngNm}^{-2}\text{s}^{-1}$  (Figure 2). The grazed grassland  $\text{N}_2\text{O}$  soil emission peak was in August with  $32.60 \text{ ngNm}^{-2}\text{s}^{-1}$ , and minimum in December 2009 with  $-5.63 \text{ ngNm}^{-2}\text{s}^{-1}$ . The flux variations were higher in the mowed grassland, with interquartile range of 26 and 20  $\text{ngNm}^{-2}\text{s}^{-1}$  from grazed grassland.

The cumulated  $\text{N}_2\text{O}$  emission were higher in the mowed grassland ( $p < 0.001$ ) with  $1596.3 \text{ gNm}^{-2}$  and  $1173.1 \text{ gNm}^{-2}$  in the grazed pasture (Figure 3).

The nitrous oxide emission factor was 0.78% and 0.55% in the grazed and mowing management system, respectively. The  $\text{N}_2\text{O}$  emission factor indicates that although the mowed grassland has higher  $\text{N}_2\text{O}$  emission, the grazed grassland emits more  $\text{N}_2\text{O}$  per kg of nitrogen applied. The grazed system emits 0.78  $\text{gNm}^{-2}$  for each gram of N applied.

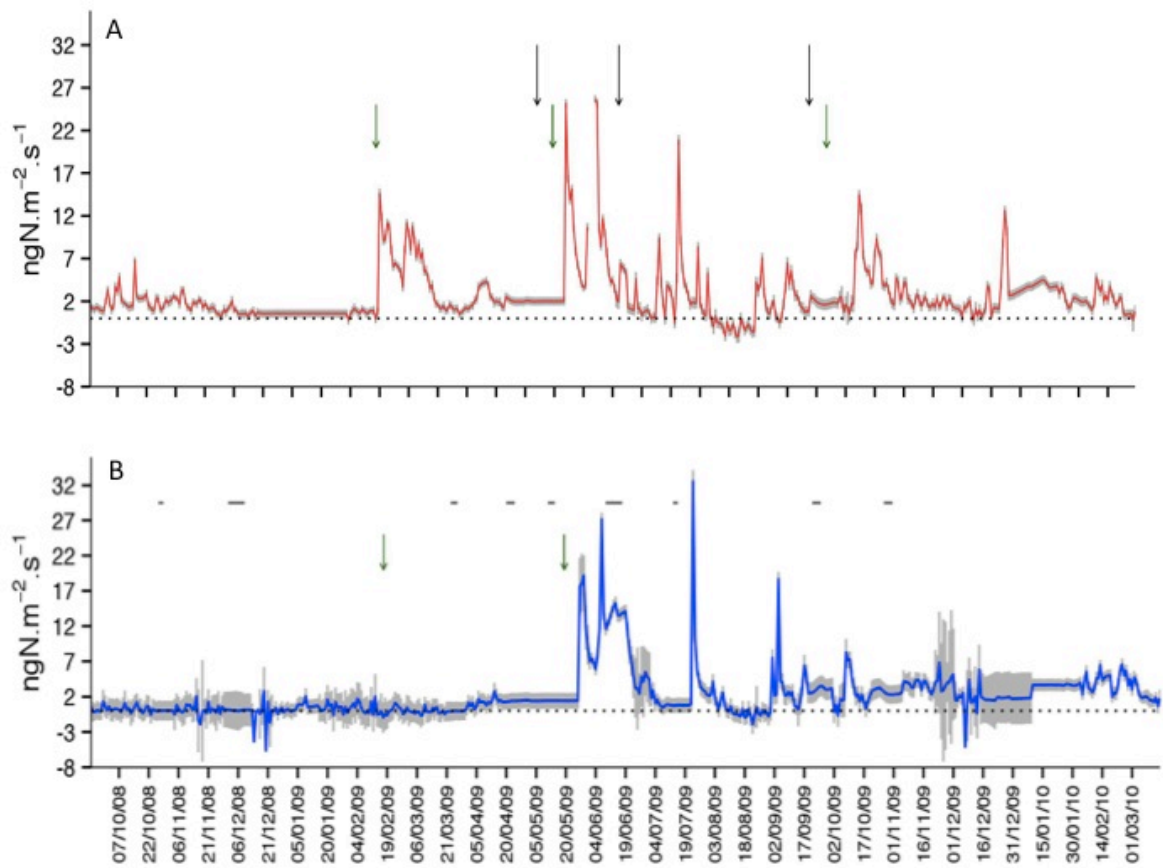


Figure 2 . Daily average  $\text{N}_2\text{O}$  flux of a mowing grassland (A) and a grazed grassland (B). Black lines are the grazing periods. Black arrows are nitrogen fertilizer and green arrows are cuts.

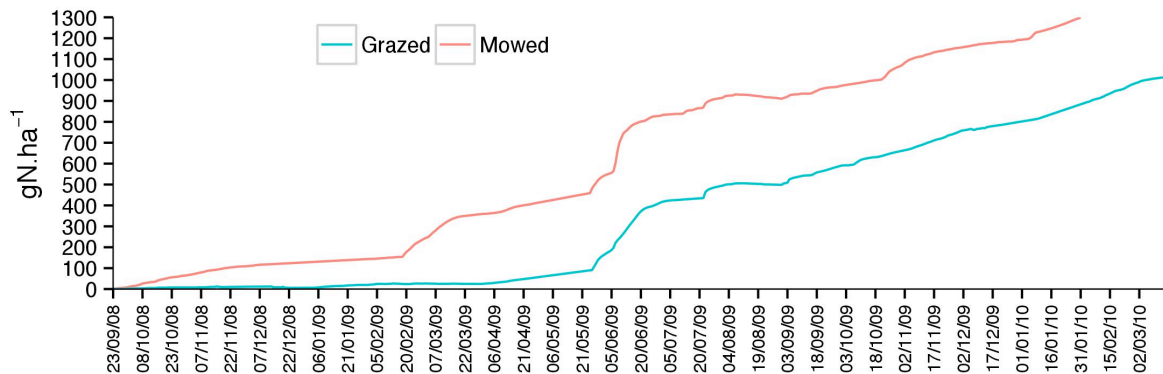


Figure 3. Cumulated N<sub>2</sub>O emission for grazed grassland and mowed grassland for 18 months.

#### 4. Discussion

In our study, most of the time the N<sub>2</sub>O fluxes measured was low, compared to other studies (Laville et al., 2011; Kammann et al., 1998). The meteorological conditions showed low level of precipitations and soil water content (<65%), indicating that nitrification was the main process producing N<sub>2</sub>O and that denitrification was restricted to anaerobic microsites due to O<sub>2</sub> inhibitory effect on this process (Linn and Doran, 1984; Meijide et al., 2009)

The N<sub>2</sub>O soil emission peaks were registered mainly after nitrogen fertilization and cutting events. Previous studies (Laville et al., 2011; Klump et al., 2011) have reported that N<sub>2</sub>O emission linearly or exponentially increases with N application, depending on the soil type, climate conditions and N fertilization rate. The increase in N<sub>2</sub>O soil emission with the N fertilization is due to increased in the soil NO<sub>3</sub> and NH<sub>4</sub> content providing substrate for microbial nitrification and denitrification to produce N<sub>2</sub>O (Liu et al., 2014). Klumpp et al. (2011) observed that some small peaks of N<sub>2</sub>O emission occurred in response to cutting events, due to cutting-induced flushes in plant rhizodeposition and soil C availability. Cutting promotes short-term N<sub>2</sub>O production, supporting the idea that cutting regime plays an important role in annual N<sub>2</sub>O-N loss from grasslands (Kammann et al., 1998). Also the mowing system received more nitrogen fertilizer than the grazed system, showing that the mineral nitrogen was a major N<sub>2</sub>O emission driver (Attard et al., 2011).

The statistical analysis showed a decreased in N<sub>2</sub>O soil emission with grazed grassland compared to mowing, in disagree with the hypothesis. According to Gao et

al. (2008), grazers can return large amounts of N to the soil through urine and feces, increasing levels of available soil N. Herbivores also increase decomposition rates by reducing C/N ratios of plants (Holland et al., 1992). Furthermore, plants often respond to defoliation by decreasing root production (Miller and Rose, 1992) that can result in reduced soil C and C/N ratios.

There were several mechanisms that could explain the increases in N<sub>2</sub>O soil emission from mowing grassland. Zou et al. (2005) established a positive linear relationship between above-ground plant biomass and N<sub>2</sub>O emissions. Kammann et al. (1998) found that decreasing the numbers of defoliations reduced N<sub>2</sub>O emissions.

Other mechanisms that alter the N<sub>2</sub>O soil emission is the compaction and probably had a important hole in this study is the soil compaction is a consequence of intensive farming if crops and animals and it occur mainly due to use of heavy machinery (Hamza and Anderson, 2005). Compaction destroys the physical soil properties by modifying porosity and impeding gas, water and nutrient movement in soil profile (Bhandral et alt., 2007). The soil compaction may impact in the N<sub>2</sub>O emission by affecting the soil aeration and indirect effect on N and C transformation (Bhandral et alt., 2007). Soil compaction reduces soil pore diameter, which in turn restricts oxygen diffusion within the soil and leads to increases the N<sub>2</sub>O production rates (van Groenigen et al., 2005).

Previous work on grasslands has shown that N<sub>2</sub>O loss due to fertilizer inputs is highly variable, ranging from 0.4% to 5.2% of N fertilizer added (Clayton et al. 1997; Rudaz et al. 1999; Abdalla et al. 2009). In our study, N<sub>2</sub>O emission factor corresponded to 0.78% to 0.55%, over a 18 months period, depending on being grazed or mowed.

## **5. Conclusion**

Nitrous oxide fluxes were found to be highly variable, with peaks of emission in response to N additions and cutting events.

Grazed pastures emits less nitrous oxide to atmosphere that mowed pastures, although grazing presented more N<sub>2</sub>O emission per kg/N applied than mowing. Indeed, N<sub>2</sub>O soil emission is dependent of soil moisture and nitrogen fertilization.

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## 5. FINAL CONCLUSIONS

The results observed in the first paper showed that exists differences in the species adaptation, production and quality to use in integrated crop livestock systems, especially under the trees shade. Despite the full sun yield is higher than in shaded areas some species showed good production and quality when growth in under the trees as *Urochloa brizantha*.

The cut intensity criterium a 50% reduction of the initial high provided a increase in leaf steam ratio, increasing the quality of the species as *Panicum maximum* cv. Aruana and *Urochloa brizantha*. Other species as *Hemarthria altissima* cv. Florida the intensity criteria needs mores studies, especially with grazing. In this experiment were used mechanical cuts. It is necessary ally the animal behavior in this kind of experiments, to understand in how the trees will affect the grazing behavior.

Also it is necessary to separate the different factors that affect the forage production in the system to better understand in how the different components (abiotic biotic factor) influence the system.

The nitrous oxide soil emissions had large variability in the systems studied, the nitrogen fertilization and the soil water content was the major drive of N<sub>2</sub>O soil emissions. The soil plowing emits large amounts of nitrogen to the atmosphere and also the N<sub>2</sub>O emission peaks is after a mechanical cut. A grazed pasture system emit less nitrous oxide to the atmosphere than a cutting pasture managing, although pastures managed with mechanical cuts use more nitrogen than grazed pasture. A grazed pasture use less nitrogen fertilization but emits more nitrous oxide per kg of nitrogen applied.

The system management knowledge is important to understand in how the production and quality of different species in different integrated production systems, as also the ecological services of this integrated crop livestock system.

A profound understanding of temporal and spatial variability of nitrous oxide fluxes between terrestrial ecosystems and the atmosphere is needed to reliably quantify these fluxes and to develop future mitigation strategies.



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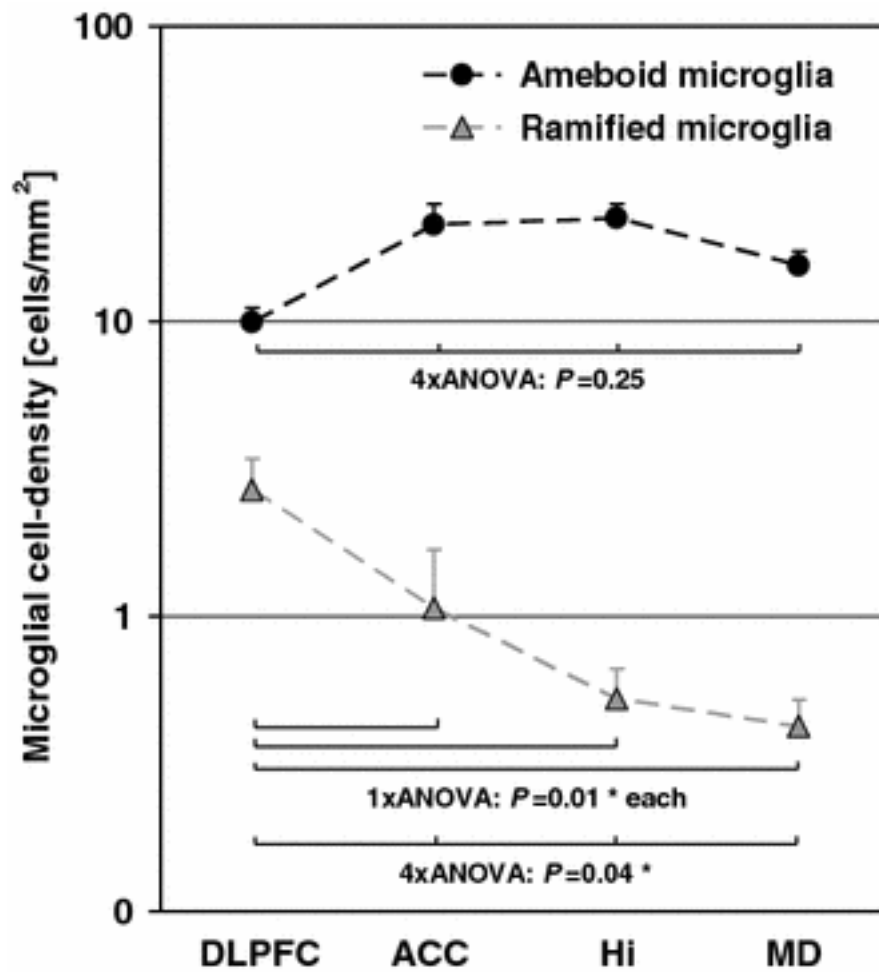
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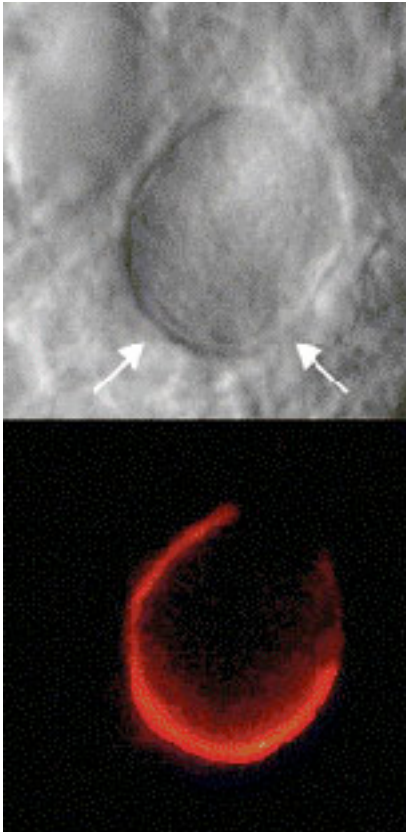
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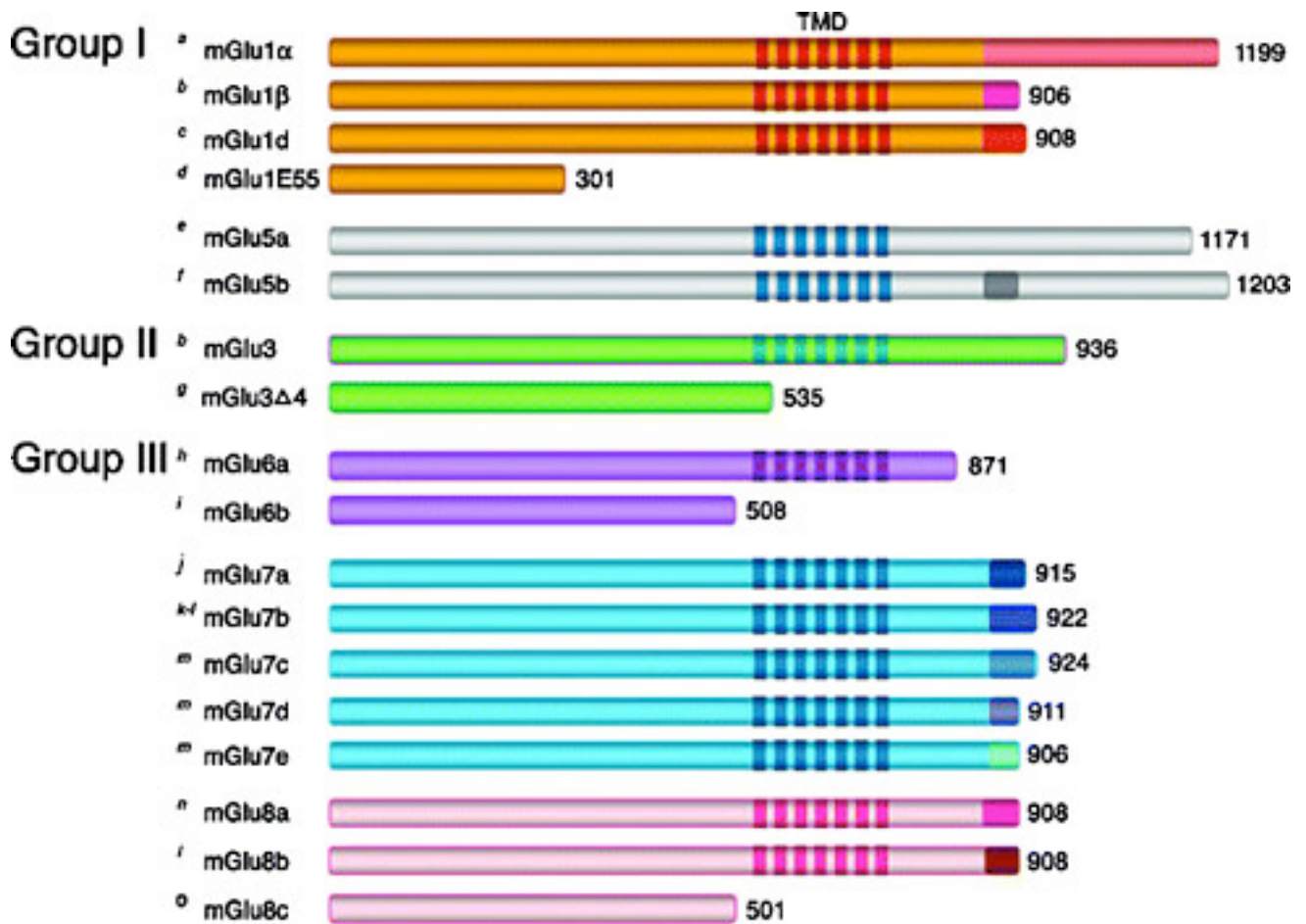
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- **Appendices:** These should be labelled with capital letters: Appendix A, Appendix B etc. Equations, figures and tables should be numbered as (A1), Fig B5 or Table C6, respectively.
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Tips for producing high-quality line graphics:



1. The first choice should be vector graphics in \*.eps or \*.pdf format.
  2. If this is not possible, a bitmap image should be saved in a "non-lossy" format, e.g. \*.png. A high resolution is recommended. It is always possible to reduce the size of the figure later.
  3. The \*.jpg format should only be used for photos. It is not suitable for sharp edges. Note that it is not advisable to convert a \*.jpg file back to \*.png.
- **Figure captions:** Each illustration should have a concise but descriptive caption. The abbreviations used in the figure must be defined, unless they are common abbreviations or have already been defined in the text. Figure captions should be included in the text file and not in the figure files.
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## References

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- **Single author papers:** chronologically, beginning with the oldest. If there is more than one paper in the same year, a letter (a, b, c) is added to the year, both in the in-text citation as well as in the reference list.
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In terms of in-text citations, the order can be based on relevance, as well as chronological or alphabetical listing, depending on the author's preference.




## Examples for Reference Sorting

In general, in-text citations can be displayed as "[...] Smith (2009) [...]", or "[...] (Smith, 2009) [...]".

Reference List	Short Citation
<b>Single author: chronologically</b>	
Smith, P.: ..., 2009.	Smith, 2009
Smith, P.: ..., 2010a.	Smith, 2010a
Smith, P.: ..., 2010b.	Smith, 2010b
<b>Co-authors: alphabetically before chronologically</b>	
Smith, P. and Brown, P.: ..., 2010.	Smith and Brown, 2010
Smith, P. and Carter, T.: ..., 2007.	Smith and Carter, 2007
Smith, P. and Carter, T.: ..., 2010a.	Smith and Carter, 2010a
Smith, P. and Carter, T.: ..., 2010b.	Smith and Carter, 2010b
Smith, P. and Thomson, A.: ..., 2005.	Smith and Thomson, 2005
<b>Team: chronologically before alphabetically</b>	
Smith, P., Thomson, A., and Carter, T.: ..., 2006.	Smith et al., 2006
Smith, P., Carter, T., and Hanson, M. B.: ..., 2008a.	Smith et al., 2008a
Smith, P., Carter, T., and Walter, N.: ..., 2008b.	Smith et al., 2008b
Smith, P., Carter, T., and Hanson, M. B.: ..., 2009.	Smith et al., 2009
Smith, P., Brown, P., and Walter, N.: ..., 2010.	Smith et al., 2010

Please do not use bold or italic writing in the reference list or for in-text citations.

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## Textual and Visual Conventions

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- **Mathematical Symbols and Formulae:** In general, mathematical symbols are typeset in italics. The most notable exceptions are function names (e.g. sin, cos), chemical formulas and physical units, which are all typeset with the normal (upright) font. Matrices are printed in bold face, and vectors in bold face italics. A range of numbers should be specified as "a to b" or "a...b". The expression "a–b" is only acceptable in cases where no confusion with "a minus b" is possible.
- **Equations:** These should be numbered sequentially with Arabic numerals in parentheses on the right-hand side, i.e. (1), (2), etc. If too long, split them accordingly. If there are chemical formulae included, i.e. reactions, please number them (R1), (R2), etc. When using WORD, the equation editor and not the graphic mode should be used under all circumstances.
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- **Abbreviations and Acronyms:** Equations should be referred to by the abbreviation "Eq." and the respective number in parentheses, e.g. "Eq. (14)". However, when the reference comes at the beginning of a sentence, the unabbreviated word "Equation" should be used, e.g.: "Equation (14) is very important for the results; however, Eq. (15) makes it clear that..." The abbreviations "Sect." and "Fig." should be used when they appear in running text and should be followed by a number unless they come at the beginning of a sentence, e.g.: "The results are depicted in Fig. 5. Figure 9 reveals that..." If acronyms or abbreviations are used throughout the article, they should be defined at first occurrence, e.g.: leaf area index (LAI), National Research Foundation (NRF). If these names or concepts are also mentioned in the abstract, they should be defined there as well.
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- **Non-English Words and Phrases:** Foreign words that have not come into general use are italicized. Words, phrases and abbreviations referenced in the Webster's are not italicized. For example, et al., cf., e.g., a priori, in situ, bremsstrahlung, and eigenvalue should not be italicized or hyphenated.