

# Plant identity and evenness affect yield and trace gas exchanges in forage mixtures

A. Ribas · R. Llurba · F. Gouriveau · N. Altimir ·  
J. Connolly · M. T. Sebastià

Received: 12 August 2014 / Accepted: 3 February 2015  
© Springer International Publishing Switzerland 2015

## Abstract

**Aims** We explore the potential effect of plant diversity on yield and greenhouse gas exchanges in forage mixtures, identifying potential co-benefits between functions.

**Methods** Using the biodiversity-ecosystem function (BEF) modelling framework (Connolly et al. 2013), we analyse results from a field experiment where the relative sown proportion of three forage species (a grass, a legume, and a non-legume forb) was varied to obtain a gradient in species proportions and evenness. The

exchange rates of N<sub>2</sub>O, CH<sub>4</sub>, NH<sub>3</sub> and CO<sub>2</sub> were measured, together with forage yield and soil inorganic N content. We conducted gas measurements 4 years after sward establishment during the expected period of highest emissions.

**Results** Results support the hypothesis that emission rates are affected by plant diversity. Sown evenness was found to decrease N<sub>2</sub>O exchange rates, and some evidence was found for a similar negative effect on CH<sub>4</sub>. Plant composition affected gas exchanges, with legume monocultures showing the highest emission rates for all gases. Diversity also increased yield and modulated NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> soil concentrations.

**Conclusions** The integrated analysis of yield and emission response to species diversity allows the identification of a range of species proportions for which both functions are optimized. Diversifying forage legume-based systems could contribute to mitigation while improving ecosystem productivity.

Responsible Editor: Elizabeth M Baggs.

**Electronic supplementary material** The online version of this article (doi:10.1007/s11104-015-2407-7) contains supplementary material, which is available to authorized users.

A. Ribas (✉) · R. Llurba · F. Gouriveau · N. Altimir ·  
M. T. Sebastià  
CTFC, Forest Sciences Centre of Catalonia, Ctra. Sant  
Llorenç de Morunys, km 2, 25280 Solsona, Spain  
e-mail: a.ribas@creaf.uab.cat

A. Ribas  
BABVE, Edifici C, Universitat Autònoma de Barcelona,  
08193 Bellaterra, Spain

A. Ribas  
CREAF, Cerdanyola del Valles, 08193 Catalonia, Spain

R. Llurba · M. T. Sebastià  
Departament HBJ, ETSEA, University of Lleida,  
25198 Lleida, Spain

J. Connolly  
UCD, School of Mathematical Sciences, Belfield, Dublin 4,  
Ireland

**Keywords** Diversity effect · Forage mixtures · GHG emissions · N<sub>2</sub>O, NH<sub>3</sub>, CO<sub>2</sub> and CH<sub>4</sub> exchange · Mitigation

## Introduction

The increasing awareness of human impact on biogeochemical cycles at global scale (IPCC 2007) has drawn attention to the need for strategies to control nutrient cycles and influence the resulting gas balance. Because of the tight link between the carbon (C) and nitrogen (N)

cycles, strategies to increase plant production and carbon dioxide (CO<sub>2</sub>) sequestration may affect, directly and indirectly, the balance of other gases, including methane (CH<sub>4</sub>) and nitrous oxide (N<sub>2</sub>O; Baggs 2011; Singh et al. 2010). Although increased N fertilization can enhance productivity and thus C sequestration, increased CO<sub>2</sub>, N<sub>2</sub>O and CH<sub>4</sub> emission that may follow N addition (Mosier et al. 1991) may offset the aimed sequestration potential (Liu and Greaver 2009). New management options must take into account the trade-offs between ecosystem functions, such as productivity and gas regulation, in order to optimize ecosystem goods and services. Most management strategies have focused on controlling the N cycling through a tighter use of reactive N in agriculture, mostly by matching N needs and supply, both at the plant and animal production levels (e.g. de Klein and Monaghan 2011; Johnson et al. 2007; Mosier 1994; Oenema et al. 2009; Snyder et al. 2009). But the challenge remains to manage agro-ecosystems to enhance self-regulating mechanisms of N cycling, and the manipulation of plant and microbial diversity may offer tools in such direction.

Although the CO<sub>2</sub> balance is the most important driver of the atmospheric global warming potential (representing c. 77 % of total annual GHG emissions, IPCC 2007), the contribution of trace gases may have important effects on the global GHG balance due to their high warming potential: CH<sub>4</sub> contributes 14.3 % and N<sub>2</sub>O 7.9 % in CO<sub>2</sub> equivalent (IPCC 2007; Sagar 2010). Agricultural systems contribute 13.5 % to total annual GHG emissions (Erisman et al. 2011; IPCC 2007) and can behave both as GHG sources and sinks (IPCC 2007). In addition, the emission of other non-greenhouse gases linked to agricultural activities, such as short-lived NH<sub>3</sub>, may affect tropospheric and stratospheric gases, having an indirect climate forcing effect (IPCC 2007).

The main natural sources of CO<sub>2</sub> are aerobic respiration and decomposition, and its major sink is global net primary productivity (Sabine 2006). Methane is generated in anoxic soils, while aerated and drained soils play a role in the CH<sub>4</sub> cycle as oxidizers (Bodelier 2011; Mosier et al. 1998). Nevertheless, methanogenesis may also take place in oxic drained soils (Conrad 2007). Soil redox potential and substrate availability determine the balance between methanogenesis and CH<sub>4</sub> oxidation.

Nitrate availability, and thus the addition of reactive N, is considered one of the main factors determining N<sub>2</sub>O emission (Erisman et al. 2011). The main N<sub>2</sub>O source in moist soils is considered to be denitrification, which is enhanced by low aeration and high labile organic C availability (Bremner 1997; Firestone et al. 1979; Wallenstein et al. 2006). Nitrous oxide can also be produced through NO<sub>3</sub><sup>-</sup> ammonification and NH<sub>3</sub> oxidation, the latter competing with CH<sub>4</sub> oxidation (Baggs 2011; Mosier et al. 1998; Steudler et al. 1989). In Mediterranean conditions both oxidation and reduction paths may be important in N<sub>2</sub>O production (Menéndez et al. 2008).

The balance between assimilation and emission of CO<sub>2</sub> and other gases may be ecosystem dependent (Macdonald et al. 2011), being greatly influenced by N and C availability and the resulting microbial (copiotrophic or oligotrophic) communities associated with nutrient pools and recalcitrance (Singh et al. 2010). Carbon assimilation is highly dependent on N availability, nevertheless, at high N levels sequestration may be offset by increased soil organic matter mineralization (Singh et al. 2010).

Plant communities, being at the interface between the soil and the atmosphere, regulate gas exchange through different pathways: first, by determining the balance between assimilation and respiration; second, by determining the quantity and quality of organic matter returned to the soil; third, by regulating the form and amount of N present in the soil throughout the year (basically through N uptake); and fourth, by affecting the soil physical conditions, which influence gas diffusivity, aeration and drainage. All these effects of plant communities determine the composition, diversity and dynamics of the microbial community, therefore behaving as a long term or distal control of emission, but also as a proximal control by influencing the immediate – physical and chemical – environment (Wallenstein et al. 2006).

Integrated approaches to improve management of the plant-soil system, considering both aboveground and belowground diversity (Baggs 2011), should shed light on possible strategies to manipulate emission (Singh et al. 2010). On the other hand, findings in the last decades confirm plant diversity effects on several ecosystem properties (Hooper et al. 2005; Zavaleta et al. 2010), including enhanced productivity (Finn et al. 2013; Hector et al. 1999; Kirwan et al. 2007); increased

soil C and N contents (Fornara and Tilman 2008; Steinbeiss et al. 2008); improved N use efficiency (Nyfeler et al. 2011; Oelmann et al. 2007); and enhanced microbial activity and diversity (Eisenhauer et al. 2011; Loranger-Merciris et al. 2006). However, little emphasis has been given to the study of plant diversity effects on emission (but see Niklaus et al. 2006 and Sun et al. 2013).

With this study we tackled this last question by measuring exchange rates of four gases ( $\text{CO}_2$ ,  $\text{CH}_4$ ,  $\text{N}_2\text{O}$  and  $\text{NH}_3$ ) along a growing season of forage swards where we manipulated plant diversity, through changes in sown plant species proportions and thus in evenness, at field conditions. We hypothesize that (i) sward composition in terms of species identity will affect the exchange rates of the measured gases; (ii) interactions between species at increased evenness levels will result in increased sward forage production and decreased GHG exchange rates; and (iii) these effects will interact with N fertilization. Our purpose was to assess if sown plant diversity can be used as a win-win strategy to increase co-benefits between yield and GHG emissions in forage crops, overcoming the trade-offs of the technologies proposed to the moment to reduce emission or/and enhance yield.

To test those hypotheses we used the methodology developed by Kirwan et al. (2007, 2009). This approach allows studying the importance of pairwise species interactions, disentangling those from individual species effects, and thus overcoming the difficulty to segregate the sampling effect from the real interaction effect when increasing the number of species in diversity manipulated communities.

## Methods

### Site description and experimental design

In spring 2008 we established a BEF (Biodiversity-Ecosystem Functioning) experiment in which we manipulated plant species composition and relative proportions, to study the plant identity and evenness effects on several ecosystem functions including forage yield, soil inorganic N content and gas exchange rates of  $\text{N}_2\text{O}$ ,  $\text{NH}_3$ ,  $\text{CO}_2$  and  $\text{CH}_4$ . The measurements presented in this paper took place in 2011, in the fourth year after

the establishment of the plant swards. During the vegetation growth period, we conducted measurements between harvests and canopy closure, close to management activities, to capture periods of highest emissions, where the potential for mitigation would also be highest. Table 1 summarises the relevant management and sampling activities during the 2011 growing season.

The experimental field was established in Castellnou d'Ossó (41° 46' N, 1° 8' E, 353 m.a.s.l.), in a semi-arid irrigated agricultural area in the Catalan Central Depression. The region is characterized by a mean annual precipitation of 428.4 mm and a mean annual temperature of 13.9 °C (data from the Catalan Meteorological Service). The field is located on an alluvial terrace, and has a carbonated soil (25–30 % carbonates) with a silt-loam texture and high water retention capacity (*Xerofluvent*) and a relatively high organic matter content (4.5 % in the first 15 cm of soil), compared with agricultural soils in the area. The main crops grown in the area are cereal and forage monocultures, with *Festuca arundinacea* and *Medicago sativa* being the most important forages.

The seed mass of the sown species was varied in different plant communities to obtain a gradient in their relative proportion resulting in a range of evenness of the communities. The plant communities included monocultures and three-species mixtures of a grass (*Festuca arundinacea*), a legume (*Medicago sativa*) and a non-legume forb (hereafter we refer to it as forb, *Cichorium intybus*), following a *simplex* design (Kirwan et al. 2007, 2009). Mixtures consisted of swards dominated in turn by each of the components (80 % sown proportion of the dominant species and 10 % of each of the minor components) and a centroid mixture with an equal proportion of each of the sown species. These seven plant communities were sown at two levels of overall sowing density, with the lowest density being 60 % of the highest, which corresponded to the density prescribed in the area of the study. Seeding rates considered appropriate in the region for each species in monoculture are 40 kg ha<sup>-1</sup> for the legume and the grass and 25 kg ha<sup>-1</sup> for the forb. This whole set of plots was sown in triplicate to allow three N fertilization levels, yielding a total of 42 (7×2×3) completely randomized plots (12 m×12 m each). The fertilization treatment started in 2011 together with emission measurements (see *Irrigation and fertilization* section).

## Vegetation and soil sampling

The plots were harvested twice in 2008 (establishment year) and every 5 to 6 weeks during the following growing seasons, with four harvests per season. At each cut, total yield was determined by harvesting 9.6 m<sup>2</sup> and weighing in-situ the fresh plant material. Plant composition was determined by cutting 0.5×0.5 m subplots to a height of 5 cm (always located at a fixed point). For every annual spring harvest, the collected material was then separated into species, oven-dried at 60 °C and weighed to estimate individual species yield. Moisture content was determined through the difference between fresh and oven-dried weight.

In 2011, soil inorganic N concentration was determined in May and June, within the second vegetation regrowth and coinciding with the two first gas exchange measurements (Table 1). The first sampling took place

just after restarting irrigation following the first harvest and previous to slurry application and, the second sampling after slurry application just before the second harvest. Six soil samples were extracted from each of the 42 plots using probes (3 cm diameter and 10 cm depth) and mixed into composite samples. Ammonium (NH<sub>4</sub><sup>+</sup>) and nitrate (NO<sub>3</sub><sup>-</sup>) concentrations were determined by colorimetric methods following Baethgen and Alley (1989) and Cataldo et al. (1975) respectively.

During emission measurements soil moistures were measured gravimetrically from three monocultures and one centroid mixture (Table 1).

## Irrigation and fertilization

The water supplied during the study was obtained from local river and well waters. Due to the fact that the experiment included fertilization levels and that it

**Table 1** Dates of harvest (H1, H2, H3), fertilization (F), soil sampling (S1, S2) and emission measurements (E1, E2, E3) along the growing period of 2011. We indicate the sampled number of plots (n) for each variable

	Dates	Samplings & fertilization	n (H/S/E)	Fertilizer N (irrigation N) kg ha <sup>-1</sup>	Irrigation (total irrig. N) mm	Precipitation mm	T <sub>air</sub> (°C)	Soil moisture (%)	
1st regrowth	11-Apr	I <sub>0</sub>			50.2 (3.7)	30			
	3-May	H1	42 (H)						
2nd regrowth	17-May	E1			8.7 (0.6)	14	18.7	15.1	
	18-May	E1	42/42/8						
	19-May	E1	(H/S/E)						
	20-May	E1+S1							
	10-Jun	F			23.3 (4.1)	311.3 (22.6)	60	23.7	20.1
	14-Jun	F+E2	42/42/8						
	15-Jun	F+E2	(H/S/E)						
17-Jun	H2+S2								
3rd regrowth	7-Jul	F+E3			240.2 (17.5)	0	25	20.5	
	8-Jul	F+E3	28 (E)	37.0 (6.0)					
	9-Jul	F+E3							
	21-Jul	H3	42 (H)		104.2 (7.6)	0			

The table includes: i. the total (ammoniacal and organic) slurry fertilizer N (corresponding to the high fertilization level) per regrowth; ii. the irrigation N, which corresponds to the nitric-N applied with irrigation water during fertilization; iii. irrigation; iv. the total accumulated nitric-N applied with cumulative irrigation amounts per period between two consecutive samplings; and v. precipitation. Irrigation and precipitation amounts were accumulated by periods (dotted squares) since restarting irrigation after winter (I<sub>0</sub>). Dashed lines indicate periods within regrowths up to each emission measurement, thus, indicated amounts of water and N refer to such periods. The table includes average 24 h air temperature during measurement periods and average soil moisture from three monocultures and one centroid mixture

required a completely randomized plot distribution, water and fertilizer were applied through an automatized sprinkler fertigation system. Fertilizer was mixed with the irrigation water at a 2 % volumetric ratio by injection. The used fertilizer was filtered pig slurry and contained 2.2 kg of total N per m<sup>3</sup>, with 48 and 52 % corresponding to the organic and inorganic (ammonium) forms respectively. Fertilization started in June 2011, within the second regrowth and was applied during two consecutive inter-harvest periods, fractionated into six applications, three per period (Table 1). For the highest N treatment 23.3 and 36.9 kg ha<sup>-1</sup> were applied in the second and third regrowth periods respectively, corresponding to an annual application of c. 100–150 kg ha<sup>-1</sup> (if a similar dose had been applied to each of the four vegetation regrowths). For the intermediate N treatment these rates were halved, and no slurry was applied in the lower N level. The same irrigation doses were applied to the three fertilization levels irrespective of the slurry applied volumes, irrigating all plots with the same amount of water that would be needed to apply the higher fertilizer doses.

In early spring, up to the first harvest, all plots received 50 mm irrigation, and during the two following regrowths all plots were irrigated with c. 330 L m<sup>-2</sup> applied per regrowth (Table 1). High N content found in the irrigation water resulted in an extra NO<sub>3</sub><sup>-</sup>-N input. We estimate that during the two regrowth periods in which emission measurements took place the extra amount of N coming from this source was around 48 kg ha<sup>-1</sup> NO<sub>3</sub><sup>-</sup>-N. Before the first measurements in May, we estimate that only c. 4 kg NO<sub>3</sub><sup>-</sup>-N had been applied with the irrigation water. At each fertigation, taking into account the slurry:water dilution ratio, the NO<sub>3</sub><sup>-</sup>-N coming from the irrigation water represented c. 14 % of the total (nitric, ammoniacal and organic) applied N.

#### Measurement of greenhouse gas exchange

In May, June and July 2011, exchange rates of carbon dioxide (CO<sub>2</sub>), nitrous oxide (N<sub>2</sub>O), methane (CH<sub>4</sub>) and ammonia (NH<sub>3</sub>) were measured in selected plant community types. In May and June, the three monocultures and the centroid mixture corresponding to the high-density level and to the high and nil N levels (4 plant community types x 1 sown density x 2 N levels) were sampled. In July, 28 plots (7×2×2) were sampled including the seven plant community types at two

densities and two N application levels (nil and high). The first fertilizer application took place just before the second gas sampling (June).

Within each month we sampled over a 2–4 day period, once or twice per day between 9:00 and 17:00 h. Consecutive measurements took place after each of the fertigation events to capture expected maximum gas emissions, starting from 1 to 12 h after slurry application (Table 1). The gas exchange measurement system consisted of opaque static chambers connected to a photoacoustic field gas-monitor (INNOVA 1412, LumaSense Technologies, Denmark) with a multiplexor system allowing for up to twelve simultaneous or sequential measurements. PVC chambers (height: 60 cm, to allow measuring during the vegetation growth period; internal diameter: 25 cm) were placed above the soil and vegetation and fitted with a rubber joint on top of PVC rings (height: 7 cm, diameter: 25 cm). The limitations (e.g., discontinuity of measurements, lack of spatial integration, system disturbance) regarding the use of chambers have been discussed widely (de Klein and Harvey 2012; Flechard et al. 2007; Hutchinson and Livingston 2002; Rochette and Eriksen-Hamel 2008; Schrier-Uijl et al. 2009; etc.), and are accounted for when interpreting the results, although it is not our purpose in this study to give absolute gas balance values for the studied system.

The rings were inserted into the ground (3–4 cm deep) several days before the measurements to ensure reasonable sealing of the system and to limit soil disturbance during measurement. The chambers were connected to the gas monitoring equipment with Teflon tubing (2 mm internal diameter). To improve spatial resolution, chambers were connected in pairs in each sampled plot using Teflon tubing and three-way valves (surface sampled area=0.098 m<sup>2</sup>); the combined exchange rates were measured by the photoacoustic equipment. Concentration measurements were compensated for cross-interferences with water vapour and the other measured gases. The detection limits of the photoacoustic gas sensor were 3.4, 0.02, 0.2 and 0.2 ppm for CO<sub>2</sub>, N<sub>2</sub>O, NH<sub>3</sub> and CH<sub>4</sub> respectively.

The recording duration was 20–40 min with the chambers closed, during which accumulated gas concentrations were measured at regular time intervals (c. every 7–10 min). Gas exchange rates were calculated from the change in concentration in the chamber over the sampling time. Since during the closure the gas concentration changed linearly with time, gas exchange values can be obtained from the slope of the linear fit to

the concentration values (Jones 1992). Positive values denote emission and negative denote uptake.

As the focus of our study was assessing the diversity effect on gas exchange, we integrated the temporal and environmental variability by averaging consecutive gas measurements, to give a single value per month, fertilization and diversity treatments. Indeed, we did not find important differences between consecutive measurements within each month that could mask gas emission peaks (individual measurements are included in the Online Resource 1, OR1- Fig. 1). Thus, to model the gas exchange response, for each month all values were averaged for sampled plots, giving 8, 8 and 28 average values of each measured gas in May, June and July respectively.

### Data analysis

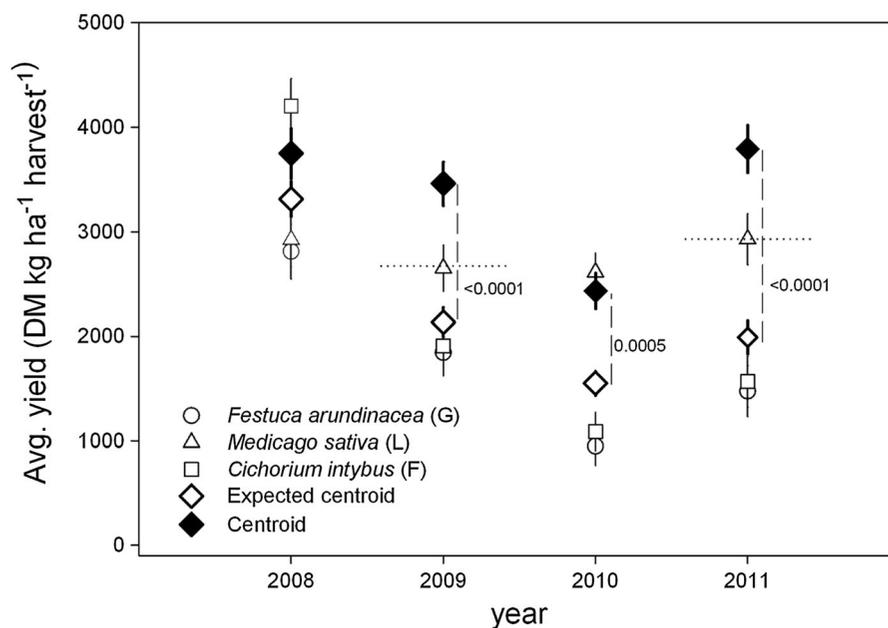
We used the methodology developed in Kirwan et al. (2007, 2009), later generalized as GDIM (General Diversity Interaction Modelling; Connolly et al. 2013), which provides a modelling framework for the diversity-ecosystem function studies. The experimental setup followed a *simplex* design (Kirwan et al. 2007,

2009), with the variables used in the model, namely species proportions and evenness, ranging between 0 and 1, and the response variable regressed against these. In this design, estimation was based on regression methods and inference was based on the residual variation around the regression model fitted (Draper and Smith 1998); thus, no replication is needed.

All studied responses, namely yield, soil inorganic N and gas exchange rates of the four sampled gases, were modelled as a linear function of the sown proportion of each species ( $P_i$ ) and a plant community evenness term ( $E$ ), plant sown density and fertilization. Evenness is as a measure of the distribution of the relative abundance of species in a community, but it also expresses the potential magnitude of species interactions. It is given by the expression (Kirwan et al. 2007):

$$E = \frac{2S}{S-1} \sum_{i < j} P_i P_j$$

where  $S$  is the community maximum plant species number and  $P_i P_j$  the species pairwise interactions. Sowing density was defined as a nominal variable with two levels, and scaled to have a zero mean, so that all other terms in the



**Fig. 1** Harvest dry matter (DM) yield (estimates ± SE) averaged per year over the experimental period (2008–2011) at average sown density and nil fertilizer application. Values correspond to the three monocultures and the centroid mixture. The expected (according to monoculture performances, ◆) and estimated (◇) centroid values are shown. The evenness effect is the difference between ◇ and ◆ (dashed line and  $p$ -value), and the transgressive

effect compares ◆ with the best performing of the three monocultures (□, ○ or △). The dotted line indicates the best performing monoculture of each year and it is shown when there is a significant transgressive overyielding. The number of averaged harvests is  $n=2$  for the establishment year (2008),  $n=4$  for 2009–2010 and  $n=3$  for 2011

model can be read as the results at average sown density. Fertilization level corresponded to total N application between two consecutive harvests, scaled to give the response per each 25 kg N applied per regrowth. A temporal factor was included when needed, corresponding to the consecutive samplings of each variable. The need of a quadratic evenness term ( $E^2$ ) in the models was also checked, and incorporated when necessary.

We used sown proportions in order to include carry-over or historical effects (Connolly et al. 2009), as these may be responsible for changes in the ecosystem biota and physical components that may impact gas emission. An alternative modeling approach could be developed using the achieved or realized species proportions instead of the intended sown proportions. Emission models using realized plant proportions are included in the Online resource 2 (OR2- Table 2). In these alternative models, we used average plant proportions since establishment (mean of the 2009–2011 species proportions in the spring harvests) in order to capture the effects of the realized plant composition across the experiment.

Repeated measures analyses of yield and soil inorganic N concentration variables were carried out across sequential yield harvests and soil samplings. Regarding exchange rates, we modelled the spring (May and June samplings) and July data separately, as they include a different number of sampled plots (8 in each spring sampling and 28 in

July). July data were fitted with a linear model as an averaged sampling. For spring data we used a repeated measures analysis but avoided including temporal effects and interactions of these and fertilization with diversity and evenness effects, as too many coefficients would be fitted with a relatively small number of data. All the statistical analyses were made using the GLM and MIXED procedures in SAS 9.1 (SAS Institute Inc., Cary, NC, USA).

This modelling approach allows the estimation of species responses in monoculture and the effect of species interactions, grouped as an evenness term, for each measurement and each level of N (Kirwan et al. 2007) and density. The evenness effect is the difference between the response of a mixture and its expected performance from the proportional contribution of each species in monoculture. We call transgressive effect the difference between the centroid mixture and the best performing (i.e. higher yielding or less emitting) monoculture.

## Results

### Sward productivity and soil inorganic N content

After the establishment of the swards (2008) we observed a positive, persistent and significant effect of sown evenness, which increased yield in

**Table 2** N<sub>2</sub>O, NH<sub>3</sub>, CO<sub>2</sub> and CH<sub>4</sub> emissions in July. Estimated coefficients ( $\pm$ SE) for the different variables included in the emission models of each studied gas, including species identity effects (Fa: *Festuca arundinacea*, Ms: *Medicago sativa*, Ci: *Cichorium*

intybus), sowing density (defined as a nominal variable with two levels with a 0 mean), fertilization (defined to give the effect per each 25 N kg ha<sup>-1</sup> applied per regrowth) and evenness. Below, we show the emission differences between each pair of monocultures

	N <sub>2</sub> O (mg m <sup>-2</sup> h <sup>-1</sup> )			NH <sub>3</sub> (mg m <sup>-2</sup> h <sup>-1</sup> )			CO <sub>2</sub> (g m <sup>-2</sup> h <sup>-1</sup> )			CH <sub>4</sub> (mg m <sup>-2</sup> h <sup>-1</sup> )		
	Coeff.	SE	<i>p</i>	Coeff.	SE	<i>p</i>	Coeff.	SE	<i>p</i>	Coeff.	SE	<i>p</i>
<i>Fa</i>	-0.15	0.049	0.005	0.36	0.20	0.083	0.99	0.184	<.0001	2.17	1.32	0.114
<i>Ms</i>	0.033	0.052	0.526	0.83	0.21	0.0006	1.85	0.193	<.0001	5.40	1.38	0.001
<i>Ci</i>	0.0020	0.052	0.970	0.36	0.21	0.098	0.81	0.193	0.0004	2.78	1.38	0.058
Density	-0.0082	0.021	0.695	0.05	0.082	0.579	0.018	0.077	0.819	0.64	0.55	0.259
Fertilization	0.045	0.028	0.128	0.07	0.11	0.520	0.20	0.105	0.075	-0.085	0.75	0.911
Evenness	-0.23	0.057	0.0007	0.21	0.23	0.368	0.75	0.212	0.002	-0.37	1.51	0.809
<i>Ms vs Fa</i>	0.19	0.062	0.007	0.47	0.25	0.070	0.86	0.23	0.0014	3.23	1.66	0.066
<i>Ms vs Ci</i>	0.031	0.064	0.627	0.48	0.25	0.075	1.04	0.24	0.0003	2.62	1.70	0.138
<i>Ci vs Fa</i>	0.16	0.062	0.021	-0.0015	0.25	0.995	-0.18	0.23	0.442	0.60	1.66	0.720

The values shown correspond to the July measurements, when 28 plots were sampled including monocultures, 1-species dominated communities and the centroid mixture at the two levels of density and at two N levels (nil and high). Note that the coefficients for the species effects give the exchange rates for the monocultures at mean density and at nil N application

mixtures over that expected from monoculture performances (Fig. 1). Across years, the legume (*Medicago sativa*) was the best performing species (Fig. 1). In the second year, the forb (*Cichorium intybus*) showed a production decrease due to its high mortality in all treatments (Fig. 1), nevertheless its biomass partially recovered, reaching yield values close to the grass (*Festuca arundinacea*) monocultures. Along the experiment, mixtures became increasingly dominated by *Medicago sativa*. The unsown species proportions remained low for most communities (<8 % for the grass, and around 1 % in mixtures and legume monocultures) being important only for the forb monocultures (14 % on average across years and 38 % in spring 2011 after the high mortality event -see Online resource 2, OR2-Table 1).

Regarding the harvests in 2011, forage yield increased with sown diversity, with a positive and significant evenness effect (Fig. 2). The yield increase in the centroid mixture relative to the expected centroid value according to monoculture performance, was 1.56, 1.86 and 2.40 respectively in the three harvests. Estimated yields for the centroid mixture were higher than the best performing species (transgressive overyielding) in two out of the three harvests (Fig. 2). In the first harvest the three monocultures gave similar values (non-significantly different; Fig. 2) but after this first harvest the legume outperformed the other two species. While in June (second harvest) the centroid mixture reached yields non-significantly different from the legume swards, in July (third harvest), this mixture outperformed the legume monoculture ( $p=0.0043$ ; Fig. 2). In 2011, density and the applied fertilization treatment did not have a significant effect on forage yield ( $p=0.36$  and  $p=0.836$  respectively).

Across diversity treatments, soil inorganic N was higher in the second (June) than in the first (May) soil sampling (Fig. 3). Although it increased in the second sampling, soil  $\text{NO}_3^-$  remained low across the two samplings, with values below  $0.3 \text{ mg NO}_3^- \text{ kg}^{-1}$  soil (Fig. 3). Within monocultures, legumes showed the highest soil  $\text{NO}_3^-$  values, significantly different from the grass ( $p=0.0002$ ) and forb ( $p=0.0011$ ) monocultures in May (Fig. 3). Regarding  $\text{NH}_4^+$ , the forb monocultures showed higher values than the other two monospecific swards (with  $p=0.0003$  and  $p=0.0002$  for the

forb-grass and forb-legume differences across samplings; Fig. 3).

Sown diversity significantly increased soil  $\text{NO}_3^-$  concentration both in May and June ( $p=0.0114$ ), with values in the centroid mixture in the range of those in the legume monoculture (non-significantly different, Fig. 3). On the other hand, diversity had a non-significant effect on  $\text{NH}_4^+$  in May, while it decreased it in June ( $p=0.0308$ ), when  $\text{NH}_4^+$  concentration reached peaks in the forb monocultures (Fig. 3). We did not detect an increase in soil inorganic N following N fertilization, with a nil effect on  $\text{NO}_3^-$ , and an effect on  $\text{NH}_4^+$  that depended on plant composition (Fig. 3). In fact, the increase in the applied slurry decreased significantly the soil ammonium content in the forb monocultures and mixtures (Fig. 3) through a negative forb  $\times$  fertilizer interaction ( $p=0.0003$ ).

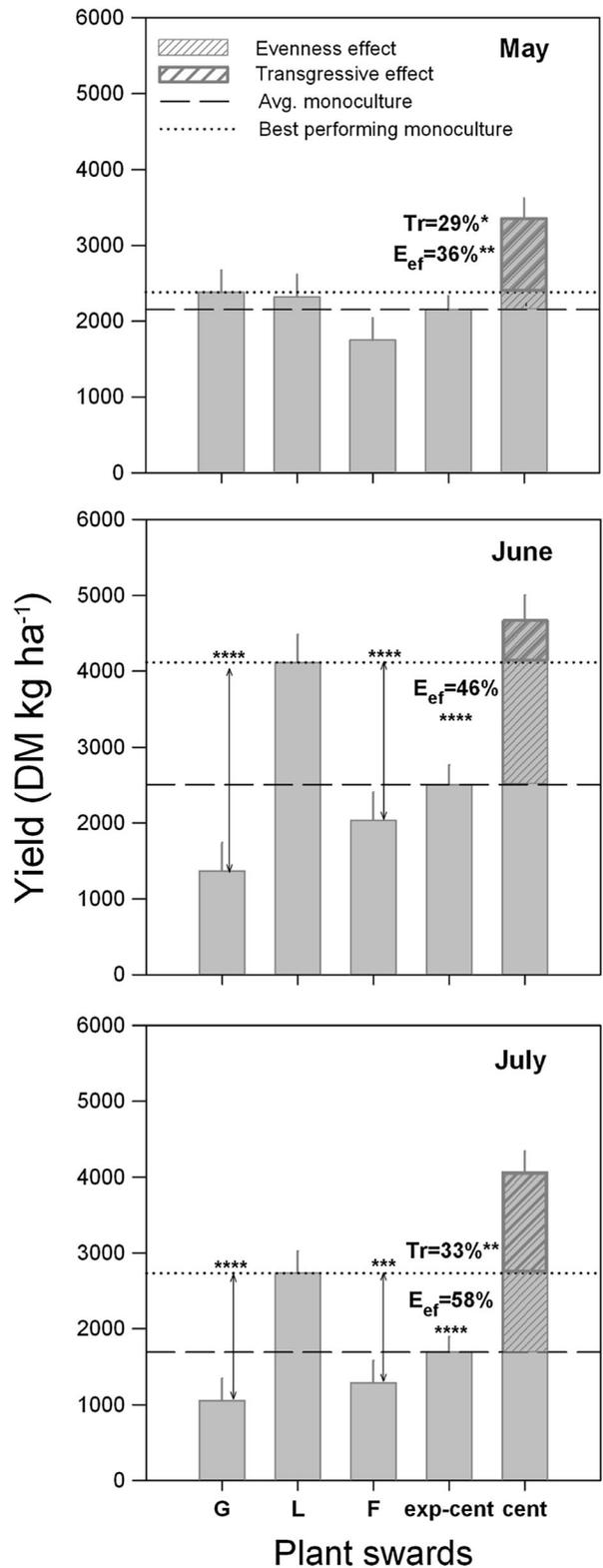
#### GHG exchange rates

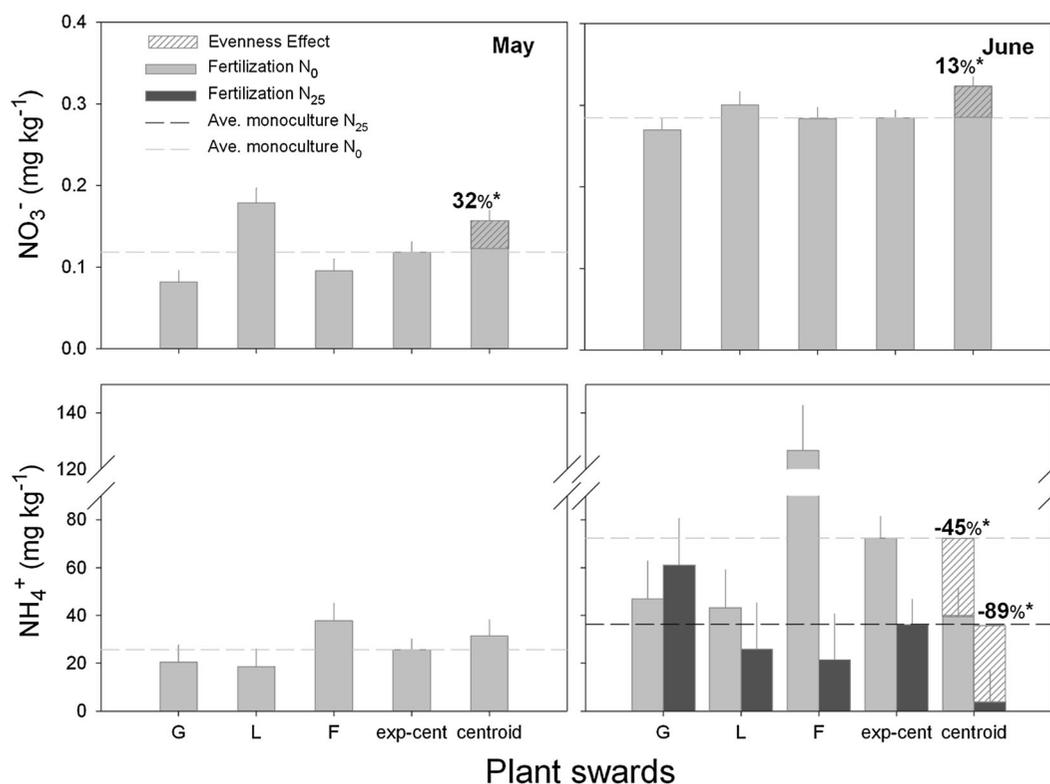
Exchange rates of the sampled gases were affected by plant diversity, and responded both to plant species identity and evenness (Tables 2 and 3, and OR2-Table 2). On the other hand, density and fertilization did not show a significant effect for any of the 4 sampled gases (Tables 2 and 3, see also OR2-Table 2 and OR1-Fig. 1 in Online resources)

Table 2 shows the results for the exchange rates in July, when all plots included in the experimental design were sampled. As a result of the significant negative diversity effect,  $\text{N}_2\text{O}$  exchange rates were reduced in mixtures relative to the expected values according to monoculture performances (Table 2, Fig. 4b). The legume and forb monocultures showed the highest  $\text{N}_2\text{O}$  exchange rates, with higher values than those found for the grass monoculture ( $p=0.007$  and  $0.021$  for the legume and the forb differences with the grass, Table 2). Regarding ammonia exchange rates, legume monocultures showed higher values as compared with the other two monospecific swards, but these differences were only marginally significant (Table 2). On the other hand, no evenness effects were detected on  $\text{NH}_3$  exchange rates (Table 2).

Diversity enhanced  $\text{CO}_2$  exchange, which corresponded to total respiration without subtracting photosynthetic assimilation (Table 2 and Fig. 4c), resulting in a higher  $\text{CO}_2$  exchange in mixtures than that expected according to monoculture exchange values.

**Fig. 2** Dry matter yield (estimates±SE) per harvest in 2011 for the three monocultures (G, grass (*Festuca arundinacea*), L, legume (*Medicago sativa*) and F, Forb (*Cichorium intybus*)) and the centroid mixture. We show values at averaged sown density and nil N application. The expected (according to monoculture performances) and estimated centroid values are shown. The difference between the two, which corresponds to the evenness effect ( $E_{\square}$ ) is indicated, relative to the estimated centroid yield (%). The transgressive overyielding effect (Tr), expressed as the difference between the estimated centroid and the best performing monoculture relative to the estimated centroid yield (%), is also indicated. Statistically significant differences are indicated as \* for  $p < 0.05$ , \*\* for  $p < 0.01$ , \*\*\* for  $p < 0.001$  and \*\*\*\* for  $p < 0.0001$





**Fig. 3** Soil nitrate ( $\text{NO}_3^-$ ; above) and ammonium ( $\text{NH}_4^+$ ; below) concentrations from 0 to 10 cm depth and for two sampling periods -May and June. The plots include estimated values $\pm$ SE, at average sown density, for the three monocultures (G, grass (*Festuca arundinacea*), L, legume (*Medicago sativa*) and F, Forb (*Cichorium intybus*)) and, the estimated and expected values (monoculture average) for the centroid mixture. Significant evenness effects are indicated, corresponding to inorganic N increase or decrease in the centroid mixture compared to the monoculture

average (expected centroid value) relative to the centroid value (when  $\Delta$ ) or, to the monoculture average (when  $\nabla$ ). Nitrogen fertilization did not affect soil  $\text{NO}_3^-$ . In the case of a significant fertilization effect (given for  $\text{NH}_4^+$  in June), values for different levels of N application ( $\text{N}_0=0$  and  $\text{N}_{25}=25$  kg N  $\text{ha}^{-1}$  per regrowth) are shown. Statistically significant differences are indicated as \* for  $p<0.05$ , \*\* for  $p<0.01$ , \*\*\* for  $p<0.001$  and \*\*\*\* for  $p<0.0001$

Regarding the different monocultures, the legume showed a higher  $\text{CO}_2$  exchange (c. 100 %) than the other two species (Table 2). In fact, the  $\text{CO}_2$  exchange for the centroid mixture was in the range of the one observed in the legume monoculture (non-significantly different).

Similarly to  $\text{CO}_2$ ,  $\text{CH}_4$  emission rates increased in legume compared to non-legume monocultures (Table 2). However, contrary to  $\text{CO}_2$ ,  $\text{CH}_4$  emission rates tended to be reduced with increased sown species evenness. Nevertheless these differences were not significant in July (Table 2).

When the average plant proportions since sward establishment were used in the models, we found similar patterns for all gases to those using the sown proportions (see Online resource 2, OR2-Table 2).

Trends observed for the spring samplings were similar to those observed in July, with the legume swards showing higher exchange rates, although differences in spring were mostly marginal (Table 3). On the other hand, no significant evenness effects were detected at that time, with the exception of a significant negative evenness effect on the methane exchange rates (Table 3).

## Discussion

The results of this study show that plant diversity produced both a positive effect on forage production and a negative effect on  $\text{N}_2\text{O}$  emission. Evidences for a negative diversity effect on  $\text{CH}_4$  emission were detected for

the spring measurements. Overall, the slurry fertilizer applied by the fertigation system did not have a significant effect neither in forage yield nor in emission (Tables 2 and 3), probably due to the fertilization technique (see section below). Thus, in our study N dynamics seemed to be driven mostly by plant composition and evenness. The high diversity effect on forage yield suggests that diversity can substitute fertilization, as concluded from other studies assessing diversity and fertilization interactions (Nyfeler et al. 2009). In our case, this fact, linked to the observed diversity effect on the modulation of gas balances, hints to the interest of increasing diversity in agroecosystems as a tool to increase co-benefits between yield and greenhouse gas exchanges, as suggested by Niklaus et al. (2006).

### Species identity effects

Leguminous crops have been reported to show enhanced emission of N (Flessa et al. 2002; Kilian and Werner 1996; Niklaus et al. 2006; Pappa et al. 2011; Rochette et al. 2004) and C (Niklaus et al. 2006). This enhancement has been generally associated with higher soil N availability (Kilian and Werner 1996; Rochette and Janzen 2005; Niklaus et al. 2006). N-rich non-legume forb crops can also produce enhanced N<sub>2</sub>O emissions after residue incorporation (Baggs et al.

2000). In our study, the forb plots had high soil NH<sub>4</sub><sup>+</sup> concentrations (Fig. 2) probably as a result of the decomposition of the forb residues following high mortality in the previous years. These high NH<sub>4</sub><sup>+</sup> concentrations might be associated with positive N<sub>2</sub>O emissions detected in the forb monocultures in the July measurements.

The used methodological approach provides a tool to compare the different plant species identity effects on gas exchanges and the impact of those relative to management practices such as fertilization. The results suggest the importance of N-rich crops in their contribution to emission relative to fertilization and, although we cannot extrapolate the data to year-round estimations, the model coefficients show an emission increase in legume vs. grass monocultures (Tables 2 and 3) that would be in the order of magnitude of the theoretical N<sub>2</sub>O-N emission produced by a N fertilization of around 150 kg N ha<sup>-1</sup> in the study period, considering an emission factor of 1.25 % (IPCC 2006).

### Diversity effects on the N cycle and GHG emission

In our study, plant diversity affected the soil inorganic N content and gas emission to a greater extent than the addition of fertilizer did. Although the soil NO<sub>3</sub><sup>-</sup> concentrations were low in the two soil samplings, this N form was enhanced with diversity, and concentrations in

**Table 3** Estimated coefficients (±SE) for the different variables included in the emission models of each studied gas (N<sub>2</sub>O, NH<sub>3</sub>, CO<sub>2</sub> and CH<sub>4</sub>), including species identity effects (*Fa*, *Festuca*

*arundinacea*; *Ms*, *Medicago sativa*; *Ci*, *Cichorium intybus*), fertilization (defined to give the effect per each 25 N kg ha<sup>-1</sup> applied per regrowth) and evenness

	N <sub>2</sub> O (mg m <sup>-2</sup> h <sup>-1</sup> )			NH <sub>3</sub> (mg m <sup>-2</sup> h <sup>-1</sup> )			CO <sub>2</sub> (g m <sup>-2</sup> h <sup>-1</sup> )			CH <sub>4</sub> (mg m <sup>-2</sup> h <sup>-1</sup> )		
	Coeff.	SE	<i>p</i>	Coeff.	SE	<i>p</i>	Coeff.	SE	<i>p</i>	Coeff.	SE	<i>p</i>
<i>Fa</i>	0.73	0.27	0.056	1.63	0.21	0.0015	1.11	0.26	0.012	4.53	1.13	0.016
<i>Ms</i>	1.73	0.27	0.003	2.38	0.21	0.0003	2.21	0.26	0.001	8.61	1.13	0.002
<i>Ci</i>	0.84	0.27	0.037	1.51	0.21	0.002	1.30	0.26	0.007	5.71	1.13	0.007
Fertilization	0.61	0.29	0.103	-0.022	0.28	0.943	0.48	0.24	0.110	-3.08	2.04	0.205
Evenness	0.62	0.31	0.121	-0.23	0.24	0.391	0.37	0.29	0.266	-4.68	1.21	0.018
<i>Ms vs Fa</i>	1.00	0.38	0.060	0.75	0.29	0.062	1.09	0.35	0.036	4.07	1.48	0.051
<i>Ms vs Ci</i>	0.89	0.38	0.082	0.87	0.29	0.041	0.91	0.35	0.061	2.89	1.48	0.122
<i>Ci vs Fa</i>	0.11	0.38	0.785	-0.12	0.29	0.698	0.19	0.35	0.624	1.18	1.48	0.469

The values correspond to the average response for May and June measurements, when eight plots were sampled including the monocultures and the centroid mixture at two N levels (nil and high). Note that the coefficients for the species effects give the exchange rates for the monocultures at nil N application. Below, we show the emission differences between each pair of monocultures

the centroid mixture were in the range of those found in the legume monoculture, that is to say, higher than average. Diversity may have increased atmospheric N fixation (Nyfeler et al. 2011) resulting in enhanced soil N content and possibly in enhanced nitrifier populations (as found in legume monocultures by Niklaus et al. 2006). This increase could produce higher N<sub>2</sub>O emissions with enhanced diversity, as it was the tendency (not significant; Table 3) found in spring.

The spring soil sampling revealed this positive diversity effect on NO<sub>3</sub><sup>-</sup> availability, but on the other hand, the amount of this N form was low when compared to NH<sub>4</sub><sup>+</sup>, which by contrast globally decreased with diversity. Moreover, cumulative N exported by the plant biomass in the three previous experimental years was also enhanced with diversity (data not shown). The diversity effect on yield observed across the three 2011 harvests could have produced a reduction in soil inorganic N content through higher plant uptake, which could be associated with the negative diversity effect on emissions found in July, as found by Niklaus et al. (2006). The NH<sub>4</sub><sup>+</sup> decrease with diversity could have also reduced CH<sub>4</sub> emission (spring, Fig. 5a), as this N form in some cases competes with CH<sub>4</sub> in its oxidation by nitrifying bacteria (Baggs 2011; Mosier et al. 1998; Steudler et al. 1989).

The effect of diversity on the N cycle eventually reflected in the gas exchange may have been also produced through different processes related to the microbial communities and to soil physical and chemical conditions. Plant diversity may have affected not only inorganic N availability (Mueller et al. 2013; Niklaus et al. 2006; Oelmann et al. 2007; Tilman et al. 1996,) and forms (Niklaus et al. 2001) present in the soil (reduced or oxidized), but also organic C availability (Fornara and Tilman 2008; Steinbeiss et al. 2008) and reductive soil conditions, all of them important determinants of the microbial oxidation and reduction processes involved in the N<sub>2</sub>O and/or CH<sub>4</sub> balances (Baggs 2011; Bremner 1997; del Prado et al. 2006; Flessa et al. 2002; Mosier et al. 1991; Wallenstein et al. 2006). The differences in NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> concentrations found according to species composition and evenness may have determined the relative importance of oxidation and reduction N<sub>2</sub>O production pathways. Moreover, a decrease in N<sub>2</sub>O emission could also derive from a complete denitrification of NO<sub>3</sub><sup>-</sup> to N<sub>2</sub> through changes in soil physical conditions (moisture) and microbial

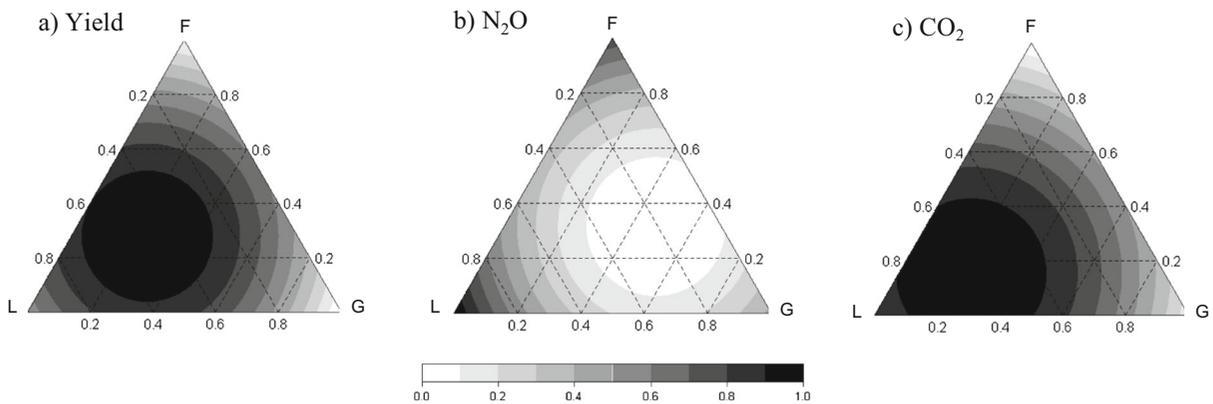
activities linked to the last reduction step. In a longer temporal scale, the microbial community composition determining gas trace production paths (methanogenesis, CH<sub>4</sub> oxidation, nitrification, denitrification, etc.) may have also been determined by plant diversity in the 3 years since sward establishment prior to the emission measurements.

Evenness increased respiration through enhanced productivity

CO<sub>2</sub> exchange rates seemed to be linked to higher plant production and activity in mixtures. Note that, in this study, due to the use of dark chambers, the CO<sub>2</sub> exchange values correspond to total dark respiration (plant and soil respiration of the enclosed system without subtracting photosynthetic assimilation). It is therefore reasonable that enhanced yield in mixed swards is followed by enhanced CO<sub>2</sub> efflux values. Indeed, if we compare the CO<sub>2</sub> gas flux and the yield contour plots (Fig. 4a and c), we find a similar pattern, which may show the high correspondence in our sampling between respiration and yield. Other respiration processes may also be included in the CO<sub>2</sub> exchange observed values apart from plant respiration, with N availability being a possible factor positively influencing total respiration (Singh et al. 2010).

Effects of fertigation conditions on emission

In our study fertilization did not produce a significant increase in GHG emission. In fact, we did not detect an increase in the soil NO<sub>3</sub><sup>-</sup> and NH<sub>4</sub><sup>+</sup> contents with fertilization, which can be the result of the fractionation, dilution and N form (around 50 % organic) of the applied fertigation. Furthermore, the slurry application was not followed by ammonia volatilization peaks, as have been commonly found (Chantigny et al. 2009), which may be due to the dilution of slurry in the irrigation water. This fertigation technique can therefore reduce NH<sub>3</sub> emission and increase infiltration (Bhandral et al. 2009) although it could also increase leaching (Velthof et al. 2009). As for other gases, NH<sub>3</sub> tended to be higher in the legume monocultures (showing marginal differences; Tables 2 and 3). These differences between crops might indicate that we captured NH<sub>3</sub> production differences (from different microbial paths) and not ammonia volatilization from the slurry application.



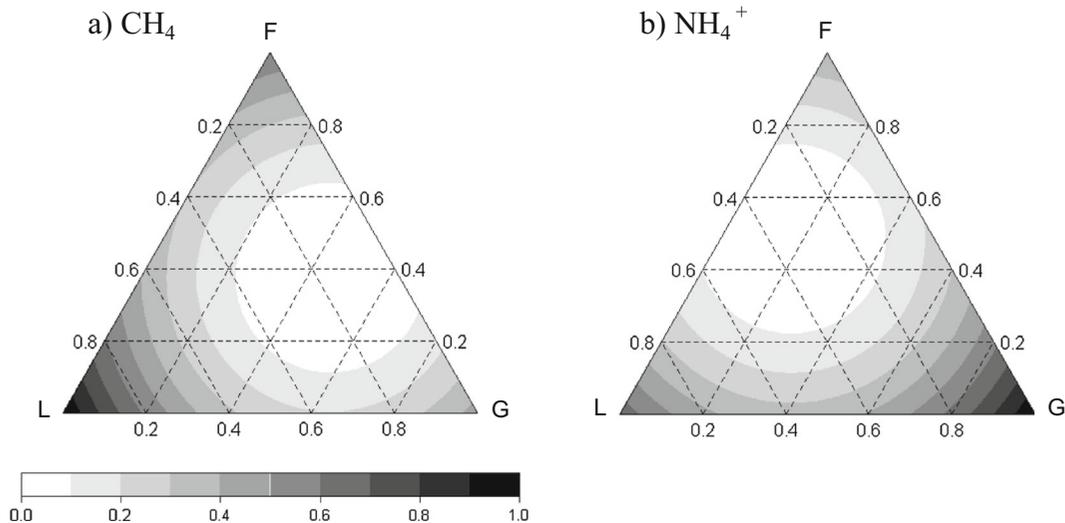
**Fig. 4** Contour plots of yield, and N<sub>2</sub>O and CO<sub>2</sub> exchange rates in July as a linear function of the three species relative proportions (Fa, *Festuca arundinacea*; Ms, *Medicago sativa* and Ci, *Cichorium intybus*) and evenness. Data correspond to the July

harvest and gas sampling. Contour plots are scaled to have a maximum of 1. Grey intensity shows a gradient from the minimum obtained value (*white*) to the highest (*black*), increasing in 10 % bands

#### Evenness increased co-benefits in ecosystem functions

The integrated effect of diversity on the studied functions, namely yield and GHG exchanges, resulted in an optimal plant composition for which yield is maximized and emission minimized. Variations of these functions as result of a change in species proportions are illustrated in contour plots (Figs. 4 and 5). Figure 4a and b shows that

the optimal function area was achieved around the centre that corresponds to the centroid mixtures (maximal evenness). According to the models in our study, only by limiting the legume proportion to 70 %, N<sub>2</sub>O emission can be kept in the range of the least emitting monoculture (grass), while yield is even higher than the best performing monoculture (legume), showing transgressive overyielding ( $p < 0.0001$ ; Fig. 4a and b). A



**Fig. 5** Contour plots of CH<sub>4</sub> exchange rates and NH<sub>4</sub><sup>+</sup> soil concentrations in spring, under a fertilization dose of 25 kg ha<sup>-1</sup> per regrowth, as a linear function of the three species relative proportions (Fa, *Festuca arundinacea*; Ms, *Medicago sativa* and Ci, *Cichorium intybus*) and evenness. In the case of CH<sub>4</sub> exchange

rates, data correspond to the spring average values (May-June) while NH<sub>4</sub><sup>+</sup> concentrations correspond to the June soil sampling. Contour plots are scaled to have a maximum of 1. Grey intensity shows a gradient from the minimum obtained value (*white*) to the highest (*black*), increasing in 10 % bands

significant transgressive under-emitting effect would be achieved when increasing grass proportion to 50 % in the mixture, with 25 % of each of the other two species ( $p=0.0451$ ). A similar trend is found for  $\text{CH}_4$  emission in spring (Fig. 5), nevertheless more data are needed to validate the observed trend.

#### Final remarks

Plant diversity has been proved to increase yield (Finn et al. 2013; Hector et al. 1999; Kirwan et al. 2007) and use reactive N more thoroughly (Nyfeler et al. 2011; Tilman et al. 1996), and can affect directly and indirectly microbial communities (Loranger-Merciris et al. 2006; Wallenstein et al. 2006). Yet, it has been scarcely proposed as a tool to control gas balances (but see Niklaus et al. 2006; Pappa et al. 2011) and strategies to decrease emission have mostly focused on improving fertilization schemes (Dittert et al. 2005; Erisman et al. 2011; IPCC 2007; Mosier 1994; Saggar 2010; etc.).

The used methodological approach provides a tool to compare the role of the different species, to segregate evenness from identity effects, and their impacts relative to management practices. Our results suggest the importance of N-rich crops in their contribution to emission relative to fertilization. On the other hand, these hint to the interest of using plant diversity as a tool to regulate nutrient cycles, as suggested by Niklaus et al. (2006). Although further data will be needed to confirm the results, these suggest that diversifying forage legume-based systems could contribute to mitigation of GHG emission while improving ecosystem productivity.

**Acknowledgments** We want to thank Rosa Teira, Camille Boubekeur, Miquel Nogueu (UdL), and Salvador Aljazairi (UB) for their help and technical support. This work was developed within projects OPS (209/PC08/3-08.2, Spanish Ministry of Environment); projects from FECYT, the Spanish Foundation for Science and Technology: CARBOAGROPAS (CGL2006-13555-C03-01/BOS), and BIOGEI (CGL-2013-49142-C2-1-R); and FLUXPYR (INTERREG IV-A POCTEFA, cofinanced by EU-ERDF, Generalitat de Catalunya and Conseil Régional Midi-Pyrénées). NA acknowledges funding through FP7/2007-2013 grant agreement n° 275855. Activities within Consolider-Ingénio MONTES SD2008-0040 and the EU COST Action 852 also contributed to the development of ideas in this paper.

#### References

- Baethgen WE, Alley MM (1989) A manual colorimetric procedure for measuring ammonium nitrogen in soil and plant digests. *Commun Soil Sci Plant Anal* 20:961–969
- Baggs EM (2011) Soil microbial sources of nitrous oxide: recent advances in knowledge, emerging challenges and future direction. *Curr Opin Environ Sustain* 3:321–327
- Baggs EM, Rees RM, Smith KA, Vinten AJA (2000) Nitrous oxide emission from soils after incorporating crop residues. *Soil Use Manag* 16:82–87
- Bhandral R, Bittman S, Kowalenko G, Buckley K, Chantigny MH, Hunt DE, Bounaix F, Friesen A (2009) Enhancing soil infiltration reduces gaseous emissions and improves N uptake from applied dairy slurry. *J Environ Qual* 38:1372–1382
- Bodelier P (2011) Interactions between nitrogenous fertilizers and methane cycling in wetland and upland. *Curr Opin Environ Sustain* 3:321–327
- Bremner JP (1997) Sources of nitrous oxide in soils. *Nutr Cycl Agroecosyst* 49:7–16
- Cataldo DA, Haroon M, Schrader LE, Youngs VL (1975) Rapid colorimetric determination of nitrate in plant tissue by nitration of salicylic acid. *Commun Soil Sci Plant Anal* 6:71–80
- Chantigny MH, MacDonald JD, Beaupré C, Rochette P, Angers DA, Massé D, Parent LE (2009) Ammonia volatilization following surface application of raw and treated liquid swine manure. *Nutr Cycl Agroecosyst* 85:275–286
- Connolly J, Finn J, Black A, Kirwan L, Brophy C, Lüscher A (2009) Effects of multi-species swards on dry matter production and the incidence of unsown species at three Irish sites. *Ir J Agric Food Res* 48:243–260
- Connolly J, Bell T, Bolger T, Brophy C, Carnus T, Finn J, Kirwan L, Isbell F, Levine J, Lüscher A, Picasso V, Roscher C, Sebastià T, Suter M, Weigelt A (2013) An improved model to predict the effects of changing biodiversity levels on ecosystem function. *J Ecol* 101:344–355
- Conrad R (2007) Microbial ecology of methanogens and methanotrophs. *Adv Agron* 96:1–63
- de Klein C, Harvey M (2012) Nitrous oxide chamber methodology guidelines. Global research alliance on agricultural greenhouse gases. Ministry for Primary Industries, Wellington, New Zealand, <http://www.globalresearchalliance.org>
- de Klein C, Monaghan R (2011) The effect of farm and catchment management on nitrogen transformations and  $\text{N}_2\text{O}$  losses from pastoral systems – can we offset the effects of future intensification? *Curr Opin Environ Sustain* 3:396–406
- del Prado A, Merino P, Estavillo JM, Pinto M, González-Murua C (2006)  $\text{N}_2\text{O}$  and NO emissions from different N sources and under a range of soil water contents. *Nutr Cycl Agroecosyst* 74:229–243
- Dittert K, Lampe C, Gasche R, Butterbach-Bahl K, Wachendorf M, Papen H, Sattelmacher B, Taube F (2005) Short-term effects of single or combined application of mineral N fertilizer and cattle slurry on the fluxes of radiatively active trace gases from grassland soil. *Soil Biol Biochem* 37:1665–1674
- Draper NR, Smith H (1998) *Applied Regression Analysis*, Wiley Series in Probability and Statistics, p 736
- Eisenhauer N, Milcu A, Sabais ACW, Bessler H, Brenner J, Engels C, Klärner B, Maraun M, Partsch S, Roscher C, Schonert F, Temperton VM, Thomisch K, Weigelt A,

- Weisser WW, Scheu S (2011) Plant diversity surpasses plant functional groups and plant productivity as driver of soil biota in the long term. *PLoS ONE* 6(1):e16055. doi:10.1371/journal.pone.0016055
- Erisman JW, Galloway J, Seitzinger S, Bleeker A, Butterbach-Bahl K (2011) Reactive nitrogen in the environment and its effect on climate change. *Curr Opin Environ Sustain* 3:281–290
- Finn JA, Kirwan L, Connolly J, Sebastià MT, Helgadottir A, Baadshaug OH, Bélanger G, Black A, Brophy C, Collins RP, Cop J, Dalmanndóttir S, Delgado I, Elgersma A, Fothergill M, Frankow-Lindberg BE, Ghesquiere A, Golinska B, Golinski P, Grieu P, Gustavsson AM, Höglind M, Huguenin-Elie O, Jorgensen M, Kadziuliene Z, Kurki P, Llubra R, Lunnan T, Porqueddu C, Suter M, Thumm U, Lüscher A (2013) Ecosystem function enhanced by combining four functional types of plant species in intensively managed grassland mixtures: a 3-year continental-scale field experiment. *J Appl Ecol* 50:365–375
- Firestone MK, Smith MS, Firestone RB, Tiedje JM (1979) Influence of nitrate, nitrite, and oxygen on the composition of the gaseous products of denitrification in soil. *Soil Sci Soc Am J* 43:1140–1144
- Flechard CR, Ambus P, Skiba U, Rees RM, Hensen A, van Amstel A, van den Pol-van DA, Soussana JF, Jones M, Clifton-Brown J, Raschi A, Horvath L, Neftel A, Jocher M, Ammann C, Leifeld J, Fuhrer J, Calanca P, Thalman E, Pilegaard K, Di Marco C, Campbell C, Nemitz E, Hargreaves KJ, Levy PE, Ball BC, Jones SK, van de Bulk WCM, Groot T, Blom M, Domingues R, Kasper G, Allard V, Ceschia E, Cellier P, Laville P, Henault C, Bizouard F, Abdalla M, Williams M, Baronti S, Berretti F, Grosz B (2007) Effects of climate and management intensity on nitrous oxide emissions in grassland systems across Europe. *Agric Ecosyst Environ* 121:135–152
- Flessa H, Ruser R, Dörsch P, Kamp T, Jimenez MA, Munch JC, Beese F (2002) Integrated evaluation of greenhouse gas emissions (CO<sub>2</sub>, CH<sub>4</sub>, N<sub>2</sub>O) from two farming systems in southern Germany. *Agric Ecosyst Environ* 91:175–189
- Fornara DA, Tilman D (2008) Plant functional composition influences rates of soil carbon and nitrogen accumulation. *J Ecol* 96:314–322
- Hector A, Schmid B, Beierkuhnlein C, Caldeira MC, Diemer M, Dimitrakopoulos PG, Finn J, Freitas H, Giller PS, Good J, Harris R, Högberg P, Huss-Danell K, Joshi J, Jumpponen A, Körner C, Leadley PW, Loreau M, Minns A, Mulder CPH, O'Donovan G, Otway SJ, Pereira JS, Prinz A, Read DJ, Scherer-Lorenzen M, Schulze ED, Siamantziouras ASD, Spehn EM, Terry AC, Troumbis AY, Woodward FI, Yachi S, Lawton JH (1999) Plant diversity and productivity experiments in European grasslands. *Science* 286:1123–1127
- Hooper DU, Chapin FS, Ewel JJ, Hector A, Inchausti P, Lavorel S, Lawton JH, Lodge DM, Loreau M, Naeem S, Schmid B, Setälä H, Symstad AJ, Vandermeer J, Wardle DA (2005) Effects of biodiversity on ecosystem functioning: a consensus of current knowledge. *Ecol Monogr* 75:3–35
- Hutchinson GL, Livingston GP (2002) Soil-atmosphere gas exchange. In: Dane JH, Topp GC (eds) *Methods of soil analysis*, 4th edn. SSSA, Madison, WI, pp 1159–1182
- IPCC (Intergovernmental Panel on Climate Change) (2006) Guidelines for national greenhouse Gas inventories. In: Eggleston HS, Buendia L, Miwa K, Ngara T, Tanabe K (eds) IPCC, national greenhouse Gas inventories programme. IGES, Japan
- Intergovernmental Panel on Climate Change (2007) Climate change: synthesis report. In: Pachauri RK, Reisinger A (eds) *Contribution of working groups I, II and III to the fourth assessment report of the intergovernmental panel on climate change*. Intergovernmental Panel on Climate Change, Geneva, Switzerland
- Johnson JM, Franzluebbers AJ, Weyers SL, Reicosky DC (2007) Agricultural opportunities to mitigate greenhouse gas emissions. *Environ Pollut* 150:107–124
- Jones H (1992) *Plants and microclimate. A quantitative approach to environmental plant physiology*. Cambridge University Press, Cambridge
- Kilian S, Werner D (1996) Enhanced denitrification in plots of N<sub>2</sub> fixing faba beans compared to plots of non-fixing legume and non legumes. *Biol Fertil Soils* 21:77–83
- Kirwan L, Lüscher A, Sebastià MT, Finn JA, Collins RP, Porqueddu C, Helgadottir A, Baadshaug OH, Brophy C, Coran C, Dalmanndóttir S, Delgado I, Elgersma A, Fothergill M, Frankow-Lindberg BE, Golinski P, Grieu P, Gustavsson AM, Höglind M, Huguenin-Elie O, Iliadis C, Jørgensen M, Kadziuliene Z, Karyotis T, Lunnan T, Malengier M, Maltoni S, Meyer V, Nyfeler D, Nykanen-Kurki P, Parente J, Smith HJ, Thumm U, Connolly J (2007) Evenness drives consistent diversity effects in intensive grassland systems across 28 European sites. *J Ecol* 95:530–539
- Kirwan L, Connolly J, Finn JA, Brophy C, Lüscher A, Nyfeler D, Sebastià MT (2009) Diversity-interaction modelling: estimating contributions of species identities and interactions to ecosystem function. *Ecology* 90:2032–2038
- Liu LL, Greaver TL (2009) A review of nitrogen enrichment effects on three biogenic GHGs: the CO<sub>2</sub> sink may be largely offset by stimulated N<sub>2</sub>O and CH<sub>4</sub> emission. *Ecol Lett* 12:1103–1117
- Loranger-Merciris G, Barthes L, Gastine A, Leadley P (2006) Rapid effects of plant species diversity and identity on soil microbial communities in experimental grassland ecosystems. *Soil Biol Biochem* 38:2336–2343
- Macdonald CA, Anderson IC, Bardgett RD, Singh BK (2011) Role of nitrogen in carbon mitigation in forest ecosystems. *Curr Opin Environ Sustain* 3:303–310
- Menéndez S, López-Bellido RJ, Benítez-Vega J, González-Murua C, López-Bellido L, Estavillo JM (2008) Long-term effect of tillage, crop rotation and N fertilization to wheat on gaseous emissions under rainfed Mediterranean conditions. *Eur J Agron* 28:559–569
- Mosier AR (1994) Nitrous oxide emissions from agricultural soils. *Fertil Res* 37:191–200
- Mosier A, Schimel DS, Valentine DW, Bronson KF, Parton WJ (1991) Methane and nitrous oxide fluxes in native, fertilized and cultivated grasslands. *Nature* 350:330–332
- Mosier AR, Duxbury JM, Freney JR, Hinemeyer O, Minami K, Johnson DE (1998) Mitigating agricultural emissions of methane. *Clim Chang* 40:39–80
- Mueller KE, Tilman D, Fornara DA, Hobbie SE (2013) Root depth distribution and the diversity-productivity relationship in a long-term grassland experiment. *Ecology* 94:787–793

- Niklaus PA, Kandeler E, Leadley PW, Schmid B, Tscherko D, Komer C (2001) A link between plant diversity, elevated CO<sub>2</sub> and soil nitrate. *Oecologia* 127:540–548
- Niklaus PA, Wardle DA, Tate KR (2006) Effects of plant species diversity and composition on nitrogen cycling and the trace gas balances of soils. *Plant Soil* 282:83–98
- Nyfelner D, Huguenin-Elie O, Suter M, Frossard E, Connolly J, Lüscher A (2009) Strong mixture effects among four species in fertilized agricultural grassland led to persistent and consistent transgressive overyielding. *J Appl Ecol* 46:683–691
- Nyfelner D, Huguenin-Elie O, Suter M, Frossard E, Lüscher A (2011) Grass-legume mixtures can yield more nitrogen than legume pure stands due to mutual stimulation of nitrogen uptake from symbiotic and non-symbiotic sources. *Agric Ecosyst Environ* 140:155–163
- Oelmann Y, Kreuziger Y, Bol R, Wilcke W (2007) Nitrate leaching in soil: Tracing the NO<sub>3</sub><sup>-</sup> sources with the help of stable N and O isotopes. *Soil Biol Biochem* 39:3024–3033
- Oenema O, Witzke HP, Klimont Z, Lesschen JP, Velthof GL (2009) Integrated assessment of promising measures to decrease nitrogen losses from agriculture in EU-27. *Agric Ecosyst Environ* 133:280–288
- Pappa VA, Rees RM, Walker RL, Baddeley JA, Watson CA (2011) Nitrous oxide emissions and nitrate leaching in an arable rotation resulting from the presence of an intercrop. *Agric Ecosyst Environ* 141:153–161
- Rochette P, Eriksen-Hamel NS (2008) Chamber measurements of soil nitrous oxide flux: are absolute values reliable? *Soil Sci Soc Am J* 72:331–342
- Rochette P, Janzen HH (2005) Towards a revised coefficient for estimating N<sub>2</sub>O emissions from legumes. *Nutr Cycl Agroecosyst* 73:171–179
- Rochette P, Angers DA, Belanger G, Chantigny M, Prévost D, Lévesque G (2004) Emissions of N<sub>2</sub>O from alfalfa and soybean crops in eastern Canada. *Soil Sci Soc Am J* 68:493–506
- Sabine CL (2006) *Global Carbon Cycle*. eLS
- Saggar S (2010) Estimation of nitrous oxide emission from ecosystems and its mitigation technologies Preface. *Agric Ecosyst Environ* 136:189–191
- Schrier-Uijl AP, Kroon PS, Hensen A, Leffelaar PA, Berendse F, Veenendaal EM (2009) Comparison of chamber and eddy covariance-based CO<sub>2</sub> and CH<sub>4</sub> emission estimates in a heterogeneous grass ecosystem on peat. *Agric Forest Meteorol* 150:825–831
- Singh BK, Bardgett RD, Smith P, Reay DS (2010) Microorganisms and climate change: Terrestrial feedbacks and mitigation options. *Nat Rev Microbiol* 8:779–790
- Snyder CS, Bruulsema TW, Jensen TL, Fixen PE (2009) Review of greenhouse gas emissions from crop production systems and fertilizer management effects. *Agric Ecosyst Environ* 133:247–266
- Steinbeiss S, Bebler H, Engels C, Temperton VM, Buchmann N, Roscher C, Kreuziger Y, Baade J, Habekost M, Cleixner G (2008) Plant diversity positively affects short-term soil carbon storage in experimental grasslands. *Global Change Biol* 14:2937–2949
- Stuedler PA, Bowden RD, Melillo JM, Aber JD (1989) Influence of nitrogen-fertilization on methane uptake in temperate forest soils. *Nature* 341:314–316
- Sun H, Zhang C, Song C, Chang S, Gu B, Chen Z, Peng C, Chang J, Ge Y (2013) The effects of plant diversity on nitrous oxide emissions in hydroponic microcosms. *Atmos Environ* 77:544–547
- Tilman D, Wedin D, Knops J (1996) Productivity and sustainability influenced by biodiversity in grassland ecosystems. *Nature* 22:703–707
- Velthof GL, Oudendag D, Witzke HR, Asman WAH, Klimont Z, Oenema O (2009) Integrated assessment of nitrogen losses from Agriculture in EU-27 using MITERRA-EUROPE. *J Environ Qual* 38:402–417
- Wallenstein MD, Myrold DD, Firestone M, Voytek M (2006) Environmental controls on denitrifying communities and denitrification rates: insights from molecular methods. *A synthesis of denitrification*. *Ecol Appl* 16:2143–2152
- Zavaleta ES, Pasari JR, Hulvey KB, Tilman GD (2010) Sustaining multiple ecosystem functions in grassland communities requires higher biodiversity. *Proc Natl Acad Sci U S A* 107:1443–1446

Online resource 1

**Journal: Plant and Soil**

**Linking effects of plant identity and species interactions on yield and trace gas exchanges in forage mixtures.**

Ribas A.<sup>1,2,3</sup>, Llurba R.<sup>1,4</sup>, Gouriveau F.<sup>1</sup>, Altimir N.<sup>1</sup>, Connolly J.<sup>5</sup> and Sebastià M.T.<sup>1,4</sup>

<sup>1</sup>CTFC, Forest Sciences Centre of Catalonia, Crta. Sant Llorenç de Morunys, km 2, 25280 Solsona, Spain

<sup>2</sup>BABVE, Edifici C, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

<sup>3</sup>CREAF, Cerdanyola del Valles 08193, Catalonia, Spain

<sup>4</sup>Depart. HBJ, ETSEA, University of Lleida, 25198 Lleida, Spain

<sup>5</sup>UCD, School of Mathematical Sciences, Belfield, Dublin 4, Ireland

Corresponding author

Àngela Ribas

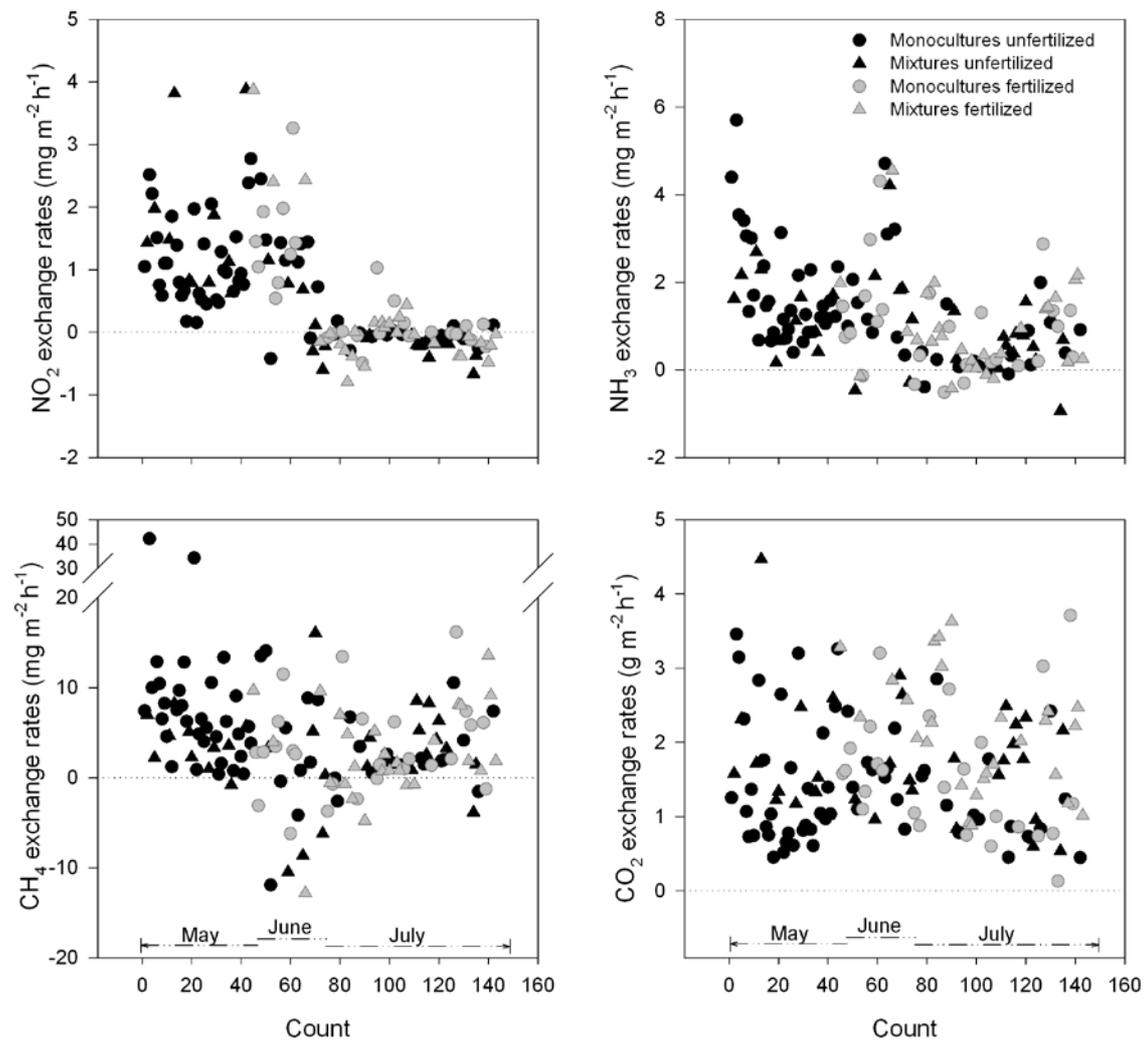
Forest Sciences Centre of Catalonia

Crta. Sant Llorenç de Morunys, km 2

25280 Solsona, Spain

e-mail: [a.ribas@creaf.uab.cat](mailto:a.ribas@creaf.uab.cat)

OR1 Figure 1.



OR 1- Figure 1: Individual gas exchange rates measured in grass, legume and forb monocultures (○) and mixtures (△). Data are distributed along the x-axis in the order in which they were taken, so that peaks or differences between measurements can be observed. The observed exchange rates show values within the same order of magnitude. Only for CH<sub>4</sub> gas emission we find two values that exceed the average (3.08 mg m<sup>-2</sup> h<sup>-1</sup>) in one order of magnitude. The figure includes data from the three measuring periods: May and June, when eight plots (6 monocultures and 2 centroid mixtures) were sampled and July, when a broader set of plant communities were included (6 monocultures, 2 centroid mixtures and 6 dominated mixtures). After the first fertilizer application in June, the measured rates include data for fertilized (light symbols) and unfertilized (dark symbols) plots.

Online resource 2

**Journal: Plant and Soil**

**Linking effects of plant identity and species interactions on yield and trace gas exchanges in forage mixtures.**

Ribas A.<sup>1,2,3</sup>, Llurba R.<sup>1,4</sup>, Gouriveau F.<sup>1</sup>, Altimir N.<sup>1</sup>, Connolly J.<sup>5</sup> and Sebastià M.T.<sup>1,4</sup>

<sup>1</sup>CTFC, Forest Sciences Centre of Catalonia, Crta. Sant Llorenç de Morunys, km 2, 25280 Solsona, Spain

<sup>2</sup>BABVE, Edifici C, Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain

<sup>3</sup>CREAF, Cerdanyola del Valles 08193, Catalonia, Spain

<sup>4</sup>Depart. HBJ, ETSEA, University of Lleida, 25198 Lleida, Spain

<sup>5</sup>UCD, School of Mathematical Sciences, Belfield, Dublin 4, Ireland

Corresponding author

Àngela Ribas

Forest Sciences Centre of Catalonia

Crta. Sant Llorenç de Morunys, km 2

25280 Solsona, Spain

e-mail: [a.ribas@creaf.uab.cat](mailto:a.ribas@creaf.uab.cat)

OR2 Table 1. Average plant composition ( $\pm$  Standard Deviation (SD)) since establishment, calculated as the mean of the 2009-2011 species proportions in the spring harvests. In the mixtures average, which includes the three dominated and the centroid mixtures, the intended proportions are 1/3 of each sown species and the evenness average would be 0.63.

	<b>Mono Fa</b>		<b>Mono Ms</b>		<b>Mono Ci</b>		<b>Mixtures</b>	
	average	SD	average	SD	average	SD	average	SD
<b>PFa</b>	0.97	0.05	0	0	0	0	0.23	0.26
<b>PMs</b>	0	0	0.99	0.01	0	0	0.61	0.28
<b>PCi</b>	0	0	0	0	0.86	0.12	0.15	0.11
<b>PUs</b>	0.03	0.47	0.01	0.01	0.14	0.12	0.01	0.01
<b>Evenness</b>	0	0	0	0	0	0	0.57	0.26

OR2 Table 2. Table OR2. Estimated coefficients ( $\pm$ SE) for the different variables included in the emission models of each studied gas ( $\text{N}_2\text{O}$ ,  $\text{NH}_3$ ,  $\text{CO}_2$  and  $\text{CH}_4$ ), including species identity effects (Fa: *Festuca arundinacea*, Ms: *Medicago sativa*, Ci: *Cichorium intybus*), sowing density (defined as a nominal variable with two levels with a 0 mean), fertilization (defined to give the effect per each 25 N kg ha<sup>-1</sup> applied per regrowth) and evenness. Realized species proportions, calculated as the mean of the 2009-2011 species proportions in the spring harvests were used in this case. The values shown correspond to the July measurements, when 28 plots were sampled including monocultures, 1-species dominated communities and the centroid mixture at the two levels of density and at two N levels (nil and high). Note that the coefficients for the species effects give the exchange rates for the monocultures at mean density and at nil N application. Below, the emission differences between each pair of monocultures are given.

	$\text{N}_2\text{O}$ (mg m <sup>-2</sup> h <sup>-1</sup> )			$\text{NH}_3$ (mg m <sup>-2</sup> h <sup>-1</sup> )			$\text{CO}_2$ (g m <sup>-2</sup> h <sup>-1</sup> )			$\text{CH}_4$ (mg m <sup>-2</sup> h <sup>-1</sup> )		
	coeff.	SE	p	coeff.	SE	p	coeff.	SE	p	coeff.	SE	p
<b>Fa</b>	-0.13	0.06	0.04	0.33	0.21	0.13	1.00	0.19	<.0001	1.54	1.41	0.29
<b>Ms</b>	-0.05	0.06	0.41	0.79	0.20	0.0006	1.83	0.17	<.0001	5.15	1.29	0.001
<b>Ci</b>	0.06	0.07	0.42	0.39	0.25	0.13	0.73	0.21	0.003	2.89	1.63	0.09
<b>density</b>	0.001	0.02	0.95	0.07	0.11	0.51	0.20	0.09	0.04	-0.06	0.72	0.94
<b>fertilization</b>	0.04	0.03	0.18	0.08	0.08	0.34	0.08	0.07	0.29	0.91	0.55	0.11
<b>Evenness</b>	-0.22	0.07	0.006	0.09	0.25	0.72	0.48	0.22	0.04	-0.74	1.64	0.66
<b>Ms vs Fa</b>	0.09	0.07	0.24	0.46	0.25	0.08	0.83	0.22	0.001	3.61	1.65	0.04
<b>Ms vs Ci</b>	-0.11	0.08	0.22	0.41	0.29	0.18	1.10	0.25	0.0003	2.26	1.92	0.25
<b>Ci vs Fa</b>	0.19	0.09	0.04	0.05	0.30	0.86	-0.28	0.26	0.30	1.35	1.98	0.50