

Article

Soil Greenhouse Gas Emissions in Different Pastures Implemented as a Management Strategy for Climate Change

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Abstract: The increase in global average temperature has significant implications for food security and agriculture production. Grass species adapted to new climate scenarios are essential for the success of livestock production. The aim of this study was to evaluate different forage species, providing animal feed during critical dry periods as an adaptation strategy for livestock grazing systems, and its implications for greenhouse gas (GHG) emissions. The field experiment was conducted during September 2015 to September 2017, with four treatments ($n = 5$, completely randomized block design), including two perennial forages, *Bromus valdivianus* (Bromus), *Lotus corniculatus* (Lotus), alone and in a mixture pasture (Br/L). As a control treatment, the forage species commonly seeded by the farmer was used (hybrid ryegrasses + Oat, Control). One-half of the plots was used for nitrous oxide (N₂O) and methane (CH₄) measurements, and the remaining half for destructive soil and forage sampling. Gas fluxes were measured using the static chambers technique. Cumulative emissions of N–N₂O, C–CH₄ and CO_{2-eq} were not affected by treatments, averaging 1.7 kg N–N₂O ha⁻¹, 3.2 kg C–CH₄ ha⁻¹ and 635.5 kg CO₂ ha⁻¹, respectively ($p > 0.05$). However, emission intensity tended to be lower for Br/L compared with other treatments ($p = 0.06$) during the second year, while pasture yield was greater for Br/L ($p < 0.05$). The control showed a greater average pasture yield (first and second years) compared to other treatments, with the highest metabolizable energy and the lowest crude protein content. Our results suggest that a mix pasture Br/L as a management strategy would promote farm adaptation, given that it favors pasture yield at critical dry periods during the year. This inclusion also reduces N–N₂O emissions from grassland soils as well as favoring C–CH₄ capture. Our study determined that N–N₂O and C–CH₄ emissions were regulated by soil variables, mainly soil moisture and soil temperature. Extension and knowledge transfer should be provided to farmers to account for potential adoption barriers, such as low short-term yield.

Keywords: climate change; emission intensity; grazing; greenhouse gas; pasture-based systems



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1. Introduction

The increase in global temperature due to greenhouse gas (GHG) accumulation in the atmosphere has significantly affected agriculture and food security, and this will be exacerbated even further in coming years [1]. During the last decade, Central and South America has suffered extreme weather events that have contributed to the vulnerability of terrestrial ecosystems resources [2]. Decreasing trends of precipitation [3,4], together with an increase in air temperature of 2–4 °C [5], have been found to reduce fodder yield [6].

In field crops and livestock production systems, direct carbon dioxide (CO₂) emissions are predominantly connected to production, while methane (CH₄) and nitrous oxide (N₂O) can potentially contribute 30% and 60% of total GHG emissions from farm activities, respectively [7]. The major impact of climate change has been observed in forage

crops and grazing systems affecting plants growth, pasture yield and quality [8–11]. The current global trend of increasing demand for livestock products is expected to continue, thereby emphasizing the importance of developing effective emission-reduction options for livestock production. Thus, several mitigation strategies have been discussed [12,13]. Additionally, ensuring an adequate availability of forage feed for animals at critical dry periods during the year becomes crucial to animal systems success, which in turn, will contribute to the planet food security.

Given the potential synergistic effects between mitigation and adaptation [12], there is a need to practice mitigation technologies with adaptation measures [6], which could alleviate the effects of climate change.

The establishment of novel pasture species, tolerant to drought and heat, and high forage yield, has been suggested as an adaptation strategy to climate change [12,13] in grazing systems. This is the case of *Bromus valdivianus*, a Chilean grass specie suitable for pasture-based livestock systems, because its high productive potential and good forage quality, even in restricted conditions of soil water content [14–16]. The drought tolerance of *B. valdivianus* is related to its deeper root system, which allows to extract water from deeper soil strata [17,18]. Replacing grassland monocultures by mixed legume–grass pastures has also been shown as a strategy for extreme weather resistance [19], as well as for mitigating N₂O emissions, due to plant morphology and biology [13,20]. Legumes can increase soil N through their nitrogen (N) fixation symbiosis with rhizobium, which has been reported to biologically fix N₂ between 32 and 115 kg ha^{−1} annually [21]. This translates into a decrease in N fertilizer use for subsequent forage seeding [22]. The legume species *Lotus corniculatus* (Lotus) is suggested to withstand an increase in temperature, with no detrimental impact on forage quality [23]. This legume is grown throughout the world, including the southern part of South America, and is one of the most drought-tolerant forage legumes typically grown as part of a mixture grass, also adapted to a wide range of soil conditions, including acidity and low fertility [24]. To the best of our knowledge, there is little information on the externalities on GHG emissions reduction from the incorporation of legume–grass mixtures in field studies.

The aim of this study was to evaluate soil GHG emissions after the seeding of different forage species-mixes implemented as a cultural management strategy that would benefit farming adaptation to climate change for livestock grazing systems. We hypothesize that the use of a mixed legume–grass pasture scheme will provide high quality animal feed at critical dry periods during the year, as well as reducing GHG emissions through mitigation as a consequence of the decrease in the use of N fertilizers.

2. Materials and Methods

2.1. Study Site

The field experiment was conducted during September 2015 to September 2017 in a commercial farm (40° 31'S, 72° 51' W, 43 m.a.s.l.), in southern Chile. The soil at the experimental site was an Andisol from the Lanco soil series (Typic Durudands; [25]), presenting low nutrient contents (Table 1). Prior to the establishment of the experiment, the soil had a natural permanent pasture used for beef grazing (*Lolium perenne* L., *Holcus lanatus* L. and *Dactylis glomerata* L.) for a period of 25 years.

Table 1. Initial (07/08/2015, $n = 4$ pseudoreplicates) and final (11/10/2017, $n = 5$) soil characterization for type of pasture (0–20 cm). Initial values \pm standard error of the mean.

Parameters	Initial	Final				SEM	p -Value
		Control	Bromus	Lotus	Br/L		
pH H ₂ O (soil:water, 1:2.5)	5.77 \pm 0.04c	5.94a	5.96a	5.91ab	5.86b	0.02	<0.01
pH CaCl ₂ (soil:CaCl ₂ , 1:2.5)	4.85 \pm 0.04b	5.13a	5.14a	5.12a	5.07a	0.02	<0.01
Organic Matter, g kg ⁻¹	234.9 \pm 7.88	229.7	237.2	235.3	242.3	4.58	0.36
Available N, mg kg ⁻¹	14.50 \pm 2.22	18.75	17.40	22.60	21.24	1.66	0.22
Olsen P, mg kg ⁻¹	5.90 \pm 0.34c	16.14b	17.09a	11.60a	13.10b	0.45	<0.01
Available S, mg kg ⁻¹	8.37 \pm 0.97a	3.0b	4.81b	3.59b	4.40b	0.67	<0.01
Exchangeable Ca, cmol (+) kg ⁻¹	2.29 \pm 0.16b	7.01a	6.99a	6.61a9a	6.99a	0.19	<0.01
Exchangeable Mg, cmol (+) kg ⁻¹	0.38 \pm 0.04b	1.06a	1.09a	1.00a	1.0.9a	0.05	<0.01
Exchangeable K, cmol (+) kg ⁻¹	0.29 \pm 0.03bc	0.43bc	0.52a	0.32ab	0.27c	0.03	<0.01
Exchangeable Na, cmol (+) kg ⁻¹	0.10 \pm 0.01ab	0.16a	0.08b	0.096b	0.13ab	0.02	<0.01
Al Saturation, % ¹	4.65 \pm 0.67a	3.90b	3.39b	1.5b	1.30b	0.27	<0.01
Bulk density, g cm ⁻³	0.68 \pm 0.007a			0.59b		0.01	<0.01
Particle density, g cm ⁻³	2.41 \pm 0.068		No sampling			-	-

¹ Al saturation: Proportion of available Al in relation to total cation content (Ca + Mg + K + Na + Al). Br/L = Bromus + Lotus. Means within a row with different letters indicate significant differences ($p < 0.05$).

2.2. Experiment Design

On 22 September 2015, four treatments were seeded on 3 \times 3 m plots corresponding to two perennial forages, *Bromus valdivianus* cv. Poker (commercial mix Bronco INIA 70% and Brominio INIA 30 %, Bromus), *Lotus corniculatus* cv. Quimey, alone and mixture pasture (Br/L), *Bromus valdivianus* + *Lotus corniculatus*. The seeding rates used were 15, 20 and 20 + 10 kg ha⁻¹, respectively. As a control treatment, the forage species commonly seeded by the farmer was used, a commercial mix called Nutrapack plus (hybrid ryegrasses cv. Belinda and Maverick GII) + Oat (*Avena sativa* L.) cv. Super Nova (35 + 10 kg ha⁻¹, respectively). Previous to seeding, soil preparation was carried out using conventional tillage (0–20 cm). Lime (1000 kg ha⁻¹, 91% CaCO₃) was applied to the soil 20 days before the experiment started, to increase the initial soil pH (Table 1). The seeding was done manually in lines spaced at 20 cm each. All treatments received a basal fertilizer application at seeding, including 30 kg N ha⁻¹ (urea, 46% N), 280 kg P₂O₅ ha⁻¹ (triple superphosphate, 46% P₂O₅), 180 kg K₂O ha⁻¹ (potassium chloride, 60% K), 40 kg S ha⁻¹ (gypsum, 18% Ca₂SO₄), 30 kg MgO ha⁻¹ (magnesium oxide, 85% MgO) and 5 kg B₂O₃ ha⁻¹ (calcium borate, 26% B₂Ca₃O₆). The N rate in further applications was carried out according to the nutritional requirement of every grass species; therefore, the treatment control received 69 kg N ha⁻¹ after the 1st forage harvest (25 November of 2015), 40 kg N ha⁻¹ during Autumn of 2016 (25 April) and 100 kg N ha⁻¹ during spring of 2016 (2 November). The Bromus treatment received N applications of 68.5 kg N ha⁻¹ during autumn of 2016 (25 April) and 40 kg N ha⁻¹ during spring of 2016 (2 November). The Lotus and Br/L did not receive additional N applications, because active fixation nodules were observed in the Lotus. During autumn of 2017, only P and K were applied in doses of 55 kg P₂O₅ ha⁻¹ (triple superphosphate, 46% P₂O₅) and 93 kg K₂O ha⁻¹ (potassium chloride, 60% K). A summary of fertilization per treatment is presented in the Table S1.

According to the experimental layout, one-half of the plots was used for N₂O and CH₄ measurements, and the remaining half for destructive soil and forage sampling. The experiment was organized on a completely randomized block design ($n = 5$).

2.3. Soil Environmental and Climatic Measurements

During gas sampling, the soil temperature (20 cm depth) and chamber temperature were measured in conjunction, using digital thermometers (Digi-Sense[®] Traceable[®], Vernon Hills, IL, United States). The daily values of air temperature (maximum, minimum, and average, °C), relative humidity, wind speed and rainfall were recorded with an auto-

matic weather station (El Cardal, Río Bueno, INIA Agrometeorological Automatic Weather Station) located at 13.1 km from the experimental site.

2.4. Soil and Plant Analysis

For initial ($n = 4$) and final ($n = 5$) soil characterization, representative soil samples (0–20 cm) were collected, for chemical and physical characterization following the methods compiled by Sadzawka et al. [26], and outlined by Rowell [27], respectively. Soil pH was measured in water and the CaCl_2 solution by potentiometry; the organic matter concentration was estimated using a modified Walkley–Black method by wet digestion. Exchangeable cations (Ca, Mg, K, and Na) and exchangeable Al were extracted with a solution of ammonium acetate ($\text{NH}_4\text{C}_2\text{H}_3\text{O}_2$) 1 M at pH 7.0 and potassium chloride (KCl) 2 M, respectively, and analyzed by atomic absorption spectrophotometry (AAS). Sulfate (SO_4^{2-}) and phosphate (PO_4^{3-}) anions were extracted in a solution of sodium bicarbonate (NaHCO_3) 0.5 M at pH 8.5 and calcium dihydrogen phosphate ($\text{Ca}(\text{H}_2\text{PO}_4)_2$) 1 M and analyzed by the Murphy and Riley method and turbidimetry, respectively.

Available soil N (N-NO_3^- and N-NH_4^+) and the soil gravimetric water content were measured (0–20 cm) every week after pasture establishment. At each sampling date, two soil samples were collected from each plot, and individually analyzed for NO_3^- and NH_4^+ using automated colorimetry (SKALAR, SA 4000, Breda, the Netherlands) and extracted with 2 M KCl [26]. The soil water content was gravimetrically determined after drying the sample at 105 °C for 24 h [27]. Bulk density was measured at the initial soil characterization and during winter and summer of 2016 and 2017. The water-filled pore space (WFPS) was calculated from the gravimetric water content data, using the results of bulk and particle densities (Table 1).

To determine the dry matter (DM) content and total yield (t DM ha^{-1}), the pasture was mown with a lawn mower when it reached 20 cm high, and weighed fresh. From this, a subsample was collected, weighted and dried at 60 °C for 48 h [28] to determine the total DM yield. The total N in pasture was determined by the Kjeldahl digestion method [28] from the same subsample.

Metabolizable energy (ME) was estimated as follows:

$$ME = 0.279 + 0.0325 * D$$

where ME (Mcal kg^{-1}) is estimated from the D, the digestible organic matter available in the dry matter (%) according to Goering and Van Soest [29].

2.5. Nitrous Oxide (N_2O) and Methane (CH_4) Flux Measurements

Fluxes of N_2O and CH_4 were measured from 22 September 2015 to 27 September 2017, using the static chambers technique [30,31]. Briefly, static chambers (25 cm diameter and 20 cm high) were placed one per plot and inserted permanently into the soil (10 cm depth) 24 h prior to the initial sampling. When sampling, chambers were closed with a hermetic PVC lid to collect gas samples in vials of 22 mL capacity. Samples were taken at 0, 20, and 40 min after closing, for four consecutive days during the first week after seeding, twice per week between the 2nd and 5th weeks, and then once a week until the next management during most of the experimental period, with the exception of summer in the first year, where particularly dry conditions (30% WFPS, Figure 1b) allowed the sampling to be carried out every two weeks. When any treatment received a new fertilization according to its recommendation, the sampling periodicity was repeated, as in the first measurement cycle. All gas samplings were carried out at 11 o'clock, as previous work shows that sampling at this time of the day provides a representative daily value (an example is provided in Table S2). Given that both soil and plants are found in experimental chambers, the quantification of CO_2 emissions was only used as a verification measure of the chambers' adequate closing.

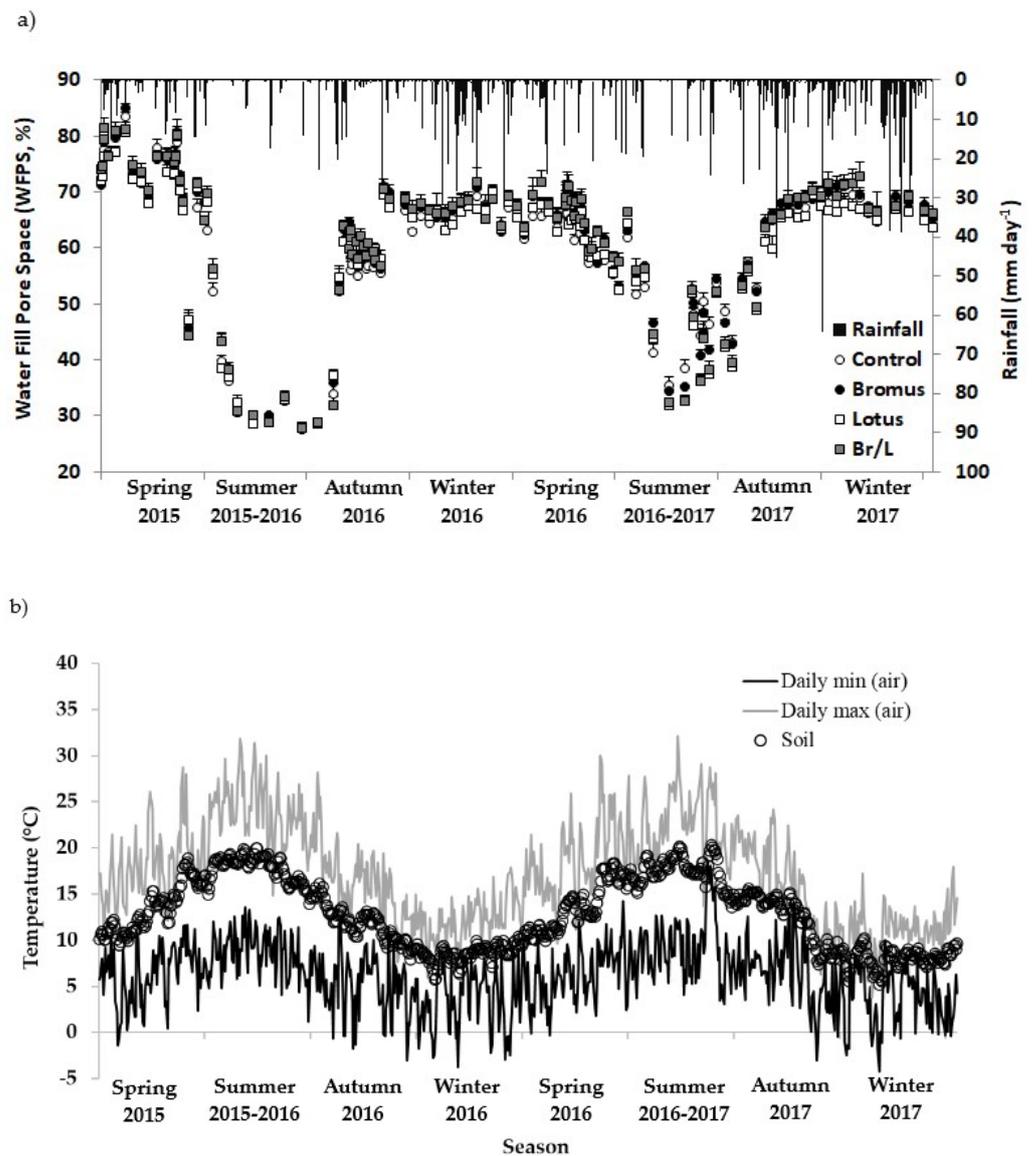


Figure 1. (a) Water-filled pore space (WFPS, %) and daily rainfall (mm, inverted in secondary axis), and (b) daily air temperature (°C) and average soil temperature (°C, 10 cm depth) during the experimental period. Original data with no interpolation.

The N₂O and CH₄ concentrations in the gas samples were determined with a gas chromatograph (Perkin Elmer[®] Precisely, Clarus 600 Model, Shelton, CT, USA) fitted with an ⁶³Ni electron capture detector (ECD) and a flame ionization detector (FID). Two Carboxen[™] 1010 PLOT columns (15 m × 0.32 mm ID, Sigma-Aldrich Co. LLC., St. Louis, MO, USA) were connected to the same injector. The oven, injector, FID and ECD temperatures were operated at 60, 260, 300 and 360 °C, respectively. The carrier gas was helium with a flow of 4 mL min⁻¹. The minimum detectable flux for the methodology was 0.011 mg N–N₂O m⁻² h⁻¹ and 0.020 mg C–CH₄ m⁻² h⁻¹.

2.6. Flux Calculations

Fluxes of N–N₂O and C–CH₄ from the static chambers were calculated from the slope of the linear increase or decrease in the three concentrations measured over the enclosure time, similar to the procedure outlined by Saggari et al. [30] and Barton et al. [32]. Flux rates were expressed on an elemental weight basis as both mg N–N₂O m⁻² h⁻¹ and g N–N₂O ha⁻¹ day⁻¹ for N₂O, and mg C–CH₄ m⁻² h⁻¹ and g C–CH₄ ha⁻¹ day⁻¹ for CH₄. The flux

rates were calculated and corrected for air temperature, atmospheric pressure and the ratio of chamber volume to surface area as follows:

$$F = \frac{\Delta C}{\Delta T} * \frac{M}{Vm} * \frac{V}{A} \quad (1)$$

where F is the flux rate ($\text{mg m}^{-2} \text{h}^{-1}$), $\Delta C/\Delta T$ is the increase in headspace concentration during the enclosure time (ppm h^{-1}), M is the molecular weight of the gas (28 for N-N₂O and 12 for C-CH₄), V_m is the pressure and temperature corrected molecular volume (L mol^{-1}), V is the volume of the measuring chamber (m^3), and A is the area of the measuring chamber (m^2), with the fraction V/A corresponding to the high of the measuring chamber.

$$Vm = \frac{R * T}{n * P} \quad (2)$$

where R is the gas constant $0.08205 \text{ atm L mol}^{-1} \text{ K}^{-1}$, T is the chamber temperature during the measurement (Kelvin), and p is the air pressure (atm) at the experimental site. Air pressure at the site was estimated from the height above sea level using a barometric equation, and n is equivalent to 1 mol of the gas.

To calculate seasonal cumulative fluxes, all calculated daily fluxes were summed according to the measurement period, before averaging across the five replicate chambers for the respective season. Days without measures were filled by linear interpolation to estimate the annual emission.

2.7. Equivalent CO₂ Emissions (CO_{2-eq})

The concept of CO_{2-eq} allows assessing the radiative forcing of different GHG's relative to the reference gas, in this case, CO₂, over a specific time horizon. The study only refers to the equivalent emissions of CH₄ and N₂O emissions from the investigated agroecosystems. Soil-borne CO₂ emissions were not taken into account for the calculation. The equivalence was calculated using the CO₂ equivalents of 28 and 265 for CH₄ and N₂O, respectively [33], and expressed in kilograms of carbon dioxide equivalents per hectare per day. The CO_{2-eq} concept allows comparing N₂O and CH₄ fluxes and assessing the relative contribution of these two gases on a reference scale, according to following formulas:

$$\text{CO}_{2-eq} = F * \frac{M_A}{M_M} * \text{GWP} \quad (3)$$

where CO_{2-eq} corresponding to the carbon dioxide equivalents ($\text{kg CO}_{2-eq} \text{ ha}^{-1} \text{ day}^{-1}$), F is the flux rate ($\text{kg ha}^{-1} \text{ day}^{-1}$), M_A is the molecular weight of the gas that come from the N and C (28 for N-N₂O and 12 for C-CH₄), M_M is the molecular weight of the gas (44 for N₂O-N and 16 for C-CH₄) and GWP is the global warming potential calculated for N₂O and CH₄ (265 and 28, respectively).

2.8. Emission Intensity

Emission intensity (EI) was calculated as the ratio of GHG emissions to agricultural production value; for this study, we considered the emission of N-N₂O ($\text{g N-N}_2\text{O ha}^{-1}$) as transformed by the GWP, and grass DM yield (kg DM ha^{-1}).

$$EI = \frac{CF}{Y} \quad (4)$$

where EI corresponds to the emission intensity, CF is the cumulative flux emission of CO_{2-eq} ($\text{kg CO}_{2-eq} \text{ ha}^{-1}$) and Y is the grass DM yield (t DM ha^{-1}).

Additionally, CO₂ emissions from CaCO₃ application were estimated using the equation reported by IPCC [33].

2.9. Statistical Analysis

Daily fluxes of N-N₂O, C-CH₄ and CO_{2-*eq*} (mg m⁻² d⁻¹) were analyzed as repeated measures in time using the MIXED procedure of SAS (PROC MIXED; SAS Institute, Cary, NC, USA). The model included the fixed effect of pasture type, season as repeated measurement, the interaction between pasture type and season and the random effects of plot and block. The pasture chemical composition was analyzed using the same model; however, the sampling time was used as a repeated measurement. Four samplings were removed from the statistical analysis, due to the lack of information for all treatments.

Annual cumulative emissions of gases, EI and pasture yield were analyzed using a mixed model, including the fixed effect of pasture type and the random effects of plot and block. An unbalanced mixed model was used to evaluate the effect of treatments on soil chemical composition at the end of the experimental period in comparison with the initial soil characterization, where the initial soil composition considered four pseudoreplicates taken at the experimental site, while the final soil composition considered five replicates (one sample per block).

For the average of daily fluxes and cumulative emissions during the full experiment, models included the fixed effect of pasture type, year as repeated measurement, interaction between pasture type and year and the random effects of plot and block.

All data met the assumption of normality and homogeneity of variance. Comparison between treatments was carried out using the Tukey test. Results were considered significant at $p < 0.05$.

A multivariate regression tree analysis was performed using R statistical software (version R-4.0.5) to identify key variables determining the N-N₂O and C-CH₄ fluxes (as dependent variable), with soil parameters (N-NO₃⁻, N-NH₄⁺, soil temperature and WFPS) as independent variables.

3. Results

3.1. Soil Environmental and Climatic Measurements

The season 2015–2016 had 75% less rainfall than the average available at the experimental site (7 years old); however, in season 2016–2017, the rainfall was 11% higher than the same period. Average ambient and soil temperatures were similar for both seasons compared to the local average, 11.5 °C and 13.3 °C, respectively (Figure 1). Most of the rainfall was concentrated in winter > autumn > spring with a dry period occurring in summer. Accordingly, low WFPS was observed in summer compared to other periods, where values were high, especially in winter and spring.

3.2. Soil and Plant Analysis

Soil

As no historical soil data management is available prior to the beginning of the experiment, it is not possible to establish the effect of prior farming management on the soil status or pasture productivity. Nevertheless, soil fertility increased towards the end of the experimental period, in response to the fertilizer application carried out (Table 1).

Olsen P increased 2.5 times, Ca concentration 3.1 times, Mg concentration 2.5 times, while Al saturation was reduced 1.9 times. Nevertheless, the S concentration decreased by 47%. Soil bulk density decreased as a result of soil movement and decompaction during cultivation.

Soil available N varied among treatments during the season in response to the differential N application rates ($p > 0.05$). Thus, N-NH₄⁺ increased in the control and Bromus treatments after urea applications (Figure 2a). The N-NO₃⁻ concentration increased due to NH₄⁺ inputs in the control and Bromus and due to soil mineralization after soil preparation and seeding in all treatments. Additionally, a slight increase was observed in the Lotus treatment in the autumn each year (March) (Figure 2b).

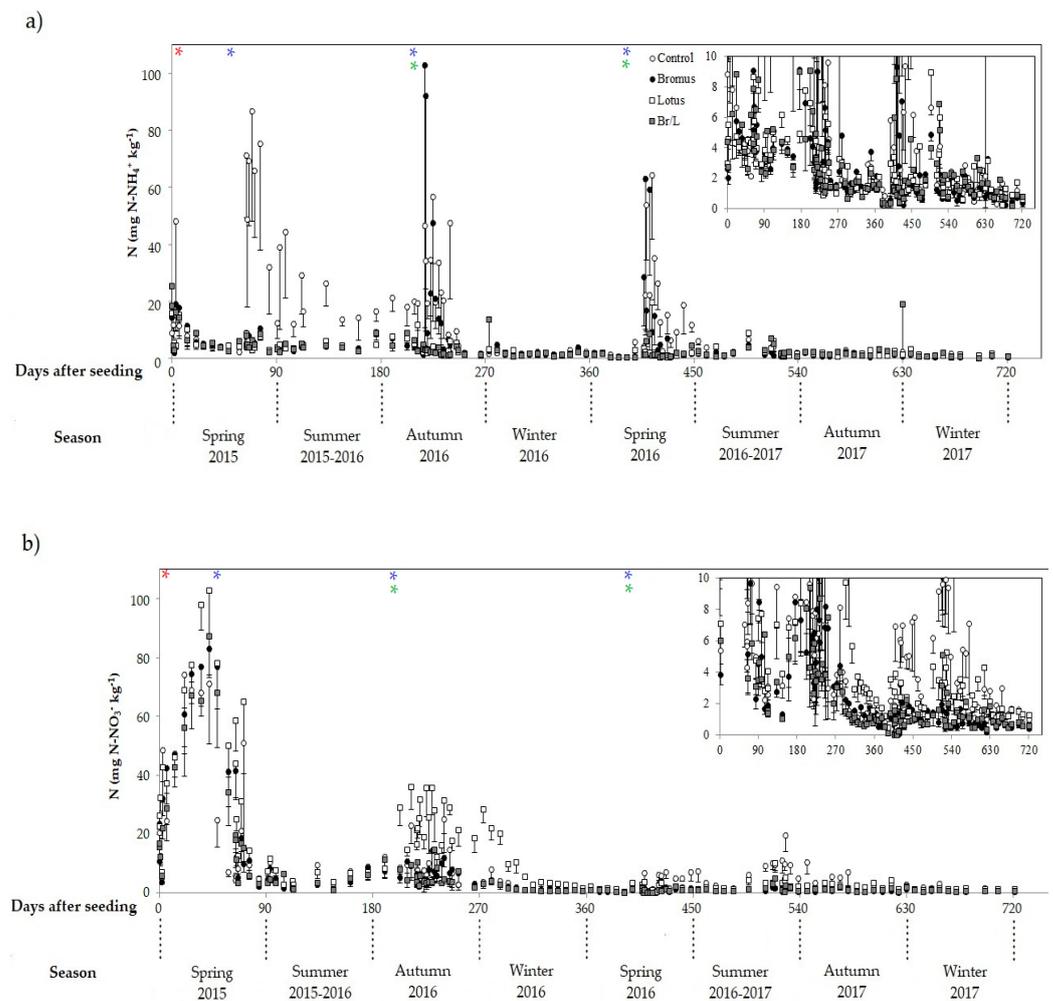


Figure 2. Concentration of soil available N as (a) ammonium ($N-NH_4^+$) and (b) nitrate ($N-NO_3^-$) ($mg\ N\ kg^{-1}$ dry soil, 0–20 cm, \pm standard error of the mean, $n = 5$) during the experimental period (September 2015–September 2017). Original data with no interpolation. Red, blue and green asterisks indicate N application to all treatments, control treatment and to Bromus, respectively. The inserts show low concentrations ($<10\ mg\ N\ kg^{-1}$ dry soil) across the experimental period.

The final soil chemical composition was similar between forage species ($p > 0.05$), with the exception of P, pH, K and Na ($p < 0.05$). The phosphorus content varied according to forage species, being greater for Bromus, followed by the control, Br/L and Lotus. The soil pH was greater for Bromus compared to the control and Lotus; however, the differences ranged between 0.05 and 0.1. The potassium concentration was 28% and 48%, greater for Bromus compared Br/L and Lotus, respectively. Finally, the Na concentration was greater for Br/L compared to Bromus.

Results of pasture chemical composition are presented in the Table 2. The N concentration was lower for the control, compared to other forage species ($p < 0.05$). However, ME and digestibility were greater for the control compared to Bromus and Lotus ($p < 0.05$). Additionally, there was an interaction between treatment and sampling time ($p < 0.01$) for all nutrients. Nitrogen concentration was higher for Br/L compared to the control in spring (of the first year (October and December)) and summer (of the second year (January)) of the experiment; however, N was greater for Lotus compared to Bromus in spring (of the first year (November)). Pasture digestibility was greater for the control than Bromus and Lotus in the summer (of the first year (January)) while it was greater for the control compared to Bromus in spring (of the second year (November)). Similarly, ME was greater for the

control, compared to Bromus for sampling for summer and spring of the first year (January and November, respectively).

Table 2. Chemical composition of forage species used in the experiment.

Parameters	Treatments (T)					p Value		
	Control	Bromus	Br/L ¹	Lotus	SEM	T	Time	TxTime
Nitrogen, %	2.44b	2.48a	2.63a	2.67a	0.06	0.04	<0.01	<0.01
Digestibility, %	78.3a	75.5b	75.9ab	74.9b	0.644	<0.01	<0.01	<0.01
ME ² , Mcal kg DM ⁻¹	2.54a	2.44b	2.45b	2.41b	0.02	<0.01	<0.01	<0.01

¹ Br/L = Bromus + Lotus; ² ME = metabolizable energy. Means within a row with different letters differ indicate significant differences ($p < 0.05$).

3.3. Fluxes of N–N₂O, C–CH₄ and CO₂ during First Year of Experiment

Results of treatment and season effects on daily fluxes of N–N₂O, C–CH₄ and CO_{2-*eq*} during first year are presented in Table 3, while average daily fluxes are shown in Figure 3. Fluxes of N–N₂O were similar among treatments ($p > 0.05$), averaging 0.422 mg N–N₂O m⁻² d⁻¹; however, fluxes were greater in spring compared with other seasons ($p < 0.01$).

Table 3. Effect of treatments and season on average daily fluxes of N–N₂O, C–CH₄ and CO_{2-*eq*} during the first and second years, and overall average.

Parameters ¹	Treatment (T)					Season (S) ³				p Value			
	Control	Bromus	Br/L	Lotus	SEM ²	Aut	Win	Spr	Sum	SEM	T	S	TxS
First year													
N ₂ O, mg N–N ₂ O m ⁻² d ⁻¹	0.404	0.419	0.404	0.460	0.031	0.320b	0.334b	0.621a	0.412b	0.027	0.54	<0.01	0.07
CH ₄ , mg C–CH ₄ m ⁻² d ⁻¹	–0.975	–1.047	–0.945	–1.023	0.038	–0.793a	–1.072b	–1.068b	–1.057b	0.029	0.25	<0.01	0.06
CO _{2-<i>eq</i>} , g CO ₂ m ⁻² d ⁻¹	0.128	0.134	0.13	0.151	0.012	0.091b	0.098b	0.227a	0.126b	0.011	0.54	<0.01	0.03
Second year													
N ₂ O, mg N–N ₂ O m ⁻² d ⁻¹	0.532a	0.50a	0.345b	0.530a	0.021	0.589b	0.287d	0.405c	0.693a	0.021	<0.01	<0.01	<0.01
CH ₄ , mg C–CH ₄ m ⁻² d ⁻¹	–0.853	–0.886	–0.909	–0.941	0.042	–0.881a	–0.841a	–0.891a	–0.976b	0.028	0.5	<0.01	0.09
CO _{2-<i>eq</i>} , g CO ₂ m ⁻² d ⁻¹	0.188	0.175	0.181	0.175	0.009	0.210b	0.098c	0.123c	0.288a	0.008	0.76	<0.01	0.77
Overall average													
N ₂ O, mg N–N ₂ O m ⁻² d ⁻¹	0.471	0.462	0.476	0.495	0.02	0.431c	0.330d	0.508b	0.635a	0.019	0.69	<0.01	0.37
CH ₄ , mg C–CH ₄ m ⁻² d ⁻¹	–0.92	–0.969	–0.932	–0.99	0.034	–0.990b	–0.959b	–0.839a	–1.021b	0.022	0.46	<0.01	0.31
CO _{2-<i>eq</i>} , g CO ₂ m ⁻² d ⁻¹	0.159	0.155	0.158	0.162	0.008	1.41c	0.100c	0.178b	0.215a	0.008	0.93	<0.01	0.56

¹ N–N₂O: nitrous oxide, C–CH₄: methane, CO₂: carbon dioxide; ² standard error of the mean ($n = 5$); ³ Aut = autumn, Win = winter, Spr = spring, Sum = summer; means within a row with different letters differ show significant differences ($p < 0.05$).

Fluxes of C–CH₄ were negative for all treatment and seasons. Fluxes of C–CH₄ were similar among treatments ($p > 0.05$), averaging -0.998 mg C–CH₄ m⁻² d⁻¹. Fluxes were greater during autumn compared with other seasons ($p < 0.01$).

Fluxes of CO_{2-*eq*} were similar among treatments, averaging 0.136 g CO_{2-*eq*} m⁻² d⁻¹. Fluxes of CO_{2-*eq*} were greater in spring compared with other seasons ($p < 0.05$). In addition, it was observed an interaction between treatment and season ($p < 0.05$, Figure 1), with CO_{2-*eq*} fluxes being greater for Lotus compared with the control and Br/L during summer ($p < 0.05$). It was also observed that CO_{2-*eq*} fluxes were greater for Lotus compared to Bromus during autumn.

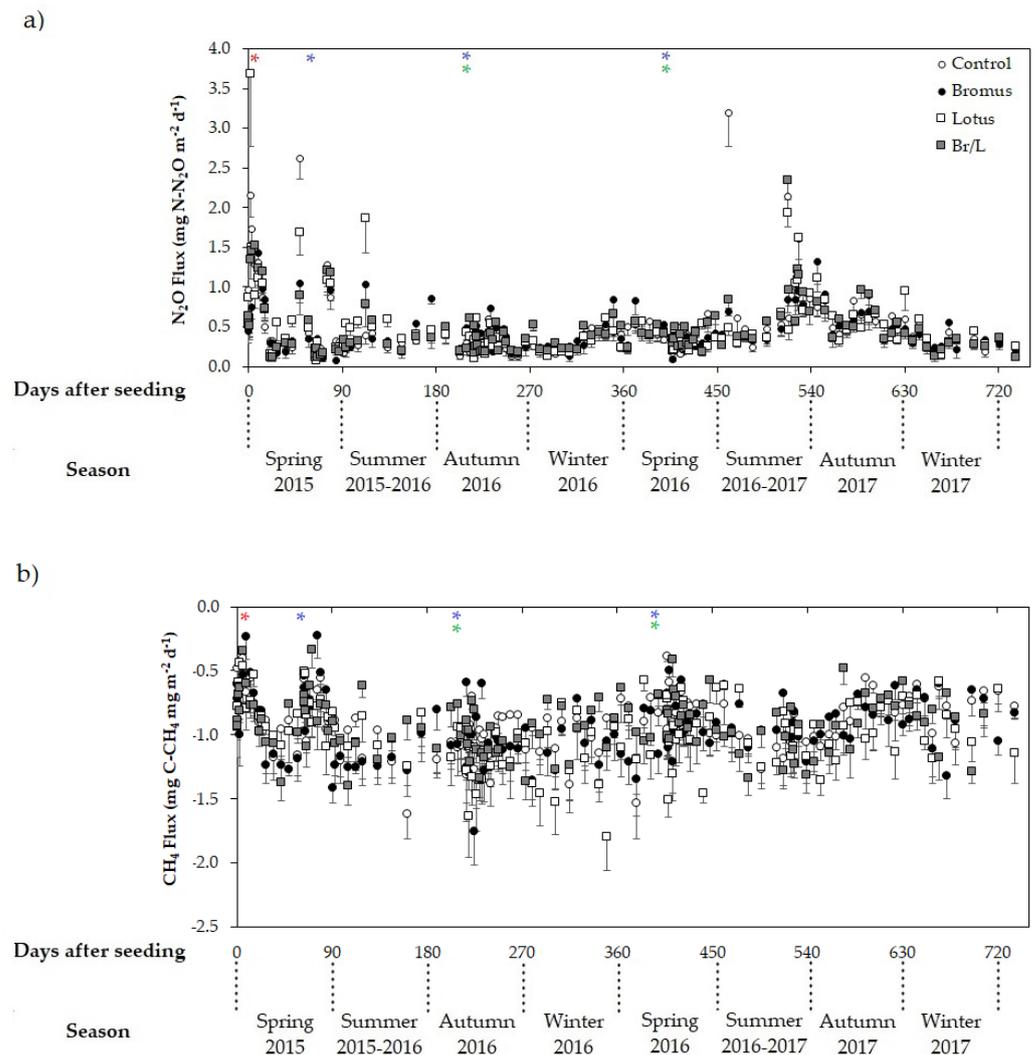


Figure 3. Daily fluxes of (a) N–N₂O and (b) C–CH₄ for the different treatments during the experimental period (September 2015–September 2017). Original data with no interpolation ($n = 5$, standard error of the mean only). Red, blue and green asterisks indicate N application to all treatments, control treatment and to Bromus, respectively.

3.4. Fluxes of N–N₂O, C–CH₄ and CO₂ during Second Year of Experiment

Results of treatment and season effects on daily fluxes of N–N₂O, C–CH₄ and CO_{2-*eq*} during second year are presented in Table 3. Seasonal effect was observed for N–N₂O, C–CH₄ and CO_{2-*eq*} fluxes ($p < 0.01$). Fluxes of N–N₂O and CO_{2-*eq*} were greater during summer compared with other seasons, while C–CH₄ fluxes were lower for summer, compared with other seasons.

Regarding treatments, fluxes of N–N₂O were lower for Br/L compared to other treatments ($p < 0.05$), while fluxes of C–CH₄ and CO_{2-*eq*} did not differ between treatments ($p > 0.05$), averaging $-0.898 \text{ mg C-CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ and $0.180 \text{ g CO}_{2-*eq*} \text{ m}^{-2} \text{ d}^{-1}$, respectively. There was an interaction between treatments and seasons for N–N₂O fluxes ($p > 0.05$). Fluxes of N–N₂O were lower for Br/L, compared to other treatments during autumn, summer and winter, while for spring, N–N₂O fluxes were similar between treatments.

3.5. Average Fluxes of N–N₂O, C–CH₄ and CO₂ during Both Years of the Experiment

Fluxes of N–N₂O, C–CH₄ and CO_{2-*eq*} did not differ between treatments ($p > 0.05$), averaging $0.48 \text{ mg N-N}_2\text{O m}^{-2} \text{ d}^{-1}$, $-0.95 \text{ mg C-CH}_4 \text{ m}^{-2} \text{ d}^{-1}$ and $0.16 \text{ g CO}_{2-*eq*} \text{ m}^{-2} \text{ d}^{-1}$, respectively (Table 3). During the first year, N–N₂O fluxes were greater in spring, compared

to other seasons ($p < 0.01$), while C-CH₄ fluxes were greater in autumn ($p < 0.05$). During the second year, emissions were greater than in the first year ($p < 0.01$), being 0.504 and 0.449 mg N-N₂O m⁻² d⁻¹, and -0.99 and -0.92 mg C-CH₄ m⁻² d⁻¹ for N-N₂O and C-CH₄, respectively, being higher for N-N₂O in summer, and for C-CH₄ in winter.

3.6. Pasture Yield, Emission Intensity and Cumulative Emissions of N-N₂O, C-CH₄ and CO₂ during the First and Second Years of the Experiment

During the first year, cumulative emissions of N-N₂O, C-CH₄ and CO_{2-eq} were not affected by treatments, averaging 1.5 kg N ha⁻¹, -3.8 kg C ha⁻¹ and 626.8 kg CO₂ ha⁻¹, respectively. Emission intensities (EI) were 25%, 41% and 73% greater for Lotus compared to Br/L, Bromus and the control, respectively ($p < 0.05$), while the pasture yield was greater for the control, compared with other treatments ($p < 0.05$) (Table 4).

Table 4. Annual cumulative emissions of N-N₂O, C-CH₄ and CO_{2-eq}, pasture yield and emission intensity (EI) of different treatments during the first and second year of experiment.

Parameters ¹	Treatments				SEM ²	p Value ³
	Control	Bromus	Br/L	Lotus		
First year						
N-N ₂ O, kg N ha ⁻¹	1.49	1.55	1.48	1.67	0.09	0.39
C-CH ₄ , kg C ha ⁻¹	-3.7	-3.89	-3.59	-3.84	0.15	0.51
CO _{2-eq} , kg C ha ⁻¹	997	1093	1074	1113	44.8	0.34
EI, kg CO _{2-eq} t ⁻¹ DM ⁻¹	88.6d	180.6c	228.9b	298.7a	11.1	<0.01
Pasture yield, t DM ha ⁻¹	11.3a	6.1b	4.8b	3.74c	0.31	<0.01
Second year						
N-N ₂ O, kg N ha ⁻¹	1.84	1.66	1.7	1.69	0.08	0.41
C-CH ₄ , kg C ha ⁻¹	-3.03	-3.17	-3.23	-3.29	0.15	0.66
CO _{2-eq} , kg CO ₂ ha ⁻¹	1125	1046	1083	1048	30.5	0.27
EI, kg CO _{2-eq} t ⁻¹ DM ⁻¹	141.1ab	123.8ab	111.4b	147.9a	7.5	0.02
Pasture yield, t DM ha ⁻¹	8.0bc	8.7ab	9.8a	7.1c	0.37	<0.01

¹ N-N₂O: nitrous oxide-N, C-CH₄: methane, CO₂: carbon dioxide; ² Standard error of the mean; ³ Trt = treatment. Br/L = Bromus + Lotus. Means within a row with different letters differ show significant differences ($p < 0.05$).

However, EI tended to be lower for Br/L compared with other treatments ($p = 0.06$) during the second year, while the pasture yield was greater for Br/L ($p < 0.05$). Cumulative emissions of N-N₂O, C-CH₄ and CO_{2-eq} were not affected by treatments, averaging 1.7 kg N-N₂O ha⁻¹, 3.2 kg C-CH₄ ha⁻¹ and 635.5 kg CO₂ ha⁻¹, respectively ($p > 0.05$).

When the two experimental years are considered together, the average cumulative emissions of N-N₂O, C-CH₄ and CO_{2-eq} were not affected by treatments ($p > 0.05$; Table 5). The emission intensities were 24%, 33% and 49% greater for Lotus compared to Br/L, Bromus and the control, respectively ($p < 0.05$). The pasture yield was greater for the control and lower for Lotus, compared to other treatments ($p < 0.05$).

Table 5. Mean annual cumulative emissions of N-N₂O, C-CH₄ and CO_{2-eq}, pasture yield and emission intensity (EI) of different pastures during the two experimental years.

Parameter ¹	Treatment (T)				SEM ²	Year (Y)		SEM	p-Values		
	Control	Bromus	Br/L	Lotus		1	2		T	Y	T*Y
N-N ₂ O, kg N ha ⁻¹	1.67	1.61	1.59	1.68	0.058	1.55	1.73	0.05	0.63	<0.01	0.24
C-CH ₄ , kg C ha ⁻¹	-3.37	-3.53	-3.41	-3.56	0.11	-3.76	-3.18	0.08	0.49	<0.01	0.62
CO _{2-eq} , kg CO ₂ ha ⁻¹	1061.4	1064.4	1078.7	1080.6	29.1	1066.9	1075.8	19.2	0.95	0.75	0.08
EI, kg CO _{2eq} t ⁻¹ DM ⁻¹	114.9c	152.2bc	170.1b	223.3a	6.7	199.2	131.1	4.73	<0.01	<0.01	<0.01
Pasture yield, t DM ha ⁻¹	9.7a	7.4b	7.2b	5.4c	0.27	6.46	8.38	0.17	<0.01	<0.01	<0.01

¹ N-N₂O: nitrous oxide-N, C-CH₄: methane, CO₂: carbon dioxide; ² Standard error of the mean; means within a row with different letters differ show significant differences ($p < 0.05$).

An interaction between treatment and year was observed for EI (first year, $p < 0.05$). The emission intensity was greater for Lotus, followed by Br/L, Bromus and control treatments, respectively. During the second year, Br/L showed a lower IE compared to other treatments. Similarly, it was observed an interaction treatment and year for pasture

yield ($p < 0.01$). In the first year, the pasture yield was greater for the control, followed by Bromus, Br/L and Lotus. However, the pasture yield was greater for Br/L compared to Bromus and Lotus in the second year.

3.7. Key Variables Determining N-N₂O and C-CH₄ Fluxes

The pasture treatment did not act as a key variable determining gas emissions. The final structure of the decision trees was similar among gases with WFPS and soil temperature as key variables (Figure 4a,b). For N-N₂O fluxes, the first splitting in the tree was controlled by the soil WFPS. When WFPS was $>53\%$ but lower than 76% , the average N-N₂O fluxes was $3.9 \text{ kg N-N}_2\text{O ha}^{-1}$; however, when WFPS $>76\%$, N-N₂O fluxes were determined by soil temperature, thus when WFPS was higher than 76% , the average N-N₂O flux was increased as the soil temperature was reduced below 13°C . On the other hand, when WFPS was less than 53% but greater than 35% , N-N₂O fluxes increased as the soil temperature rose over 15°C . In the final tree structure for C-CH₄ fluxes, when WFPS was lower than 68% but greater than 39% , the C-CH₄ capture was controlled by soil temperature. However, when WFPS was lower than 39% , the average C-CH₄ fluxes was $-11 \text{ kg C-CH}_4 \text{ ha}^{-1} \text{ d}^{-1}$, being only controlled by WFPS. Therefore, N-N₂O emissions and C-CH₄ capture on temperate pastures of Southern Chile is mainly determined by the soil WFPS, followed by soil temperature.

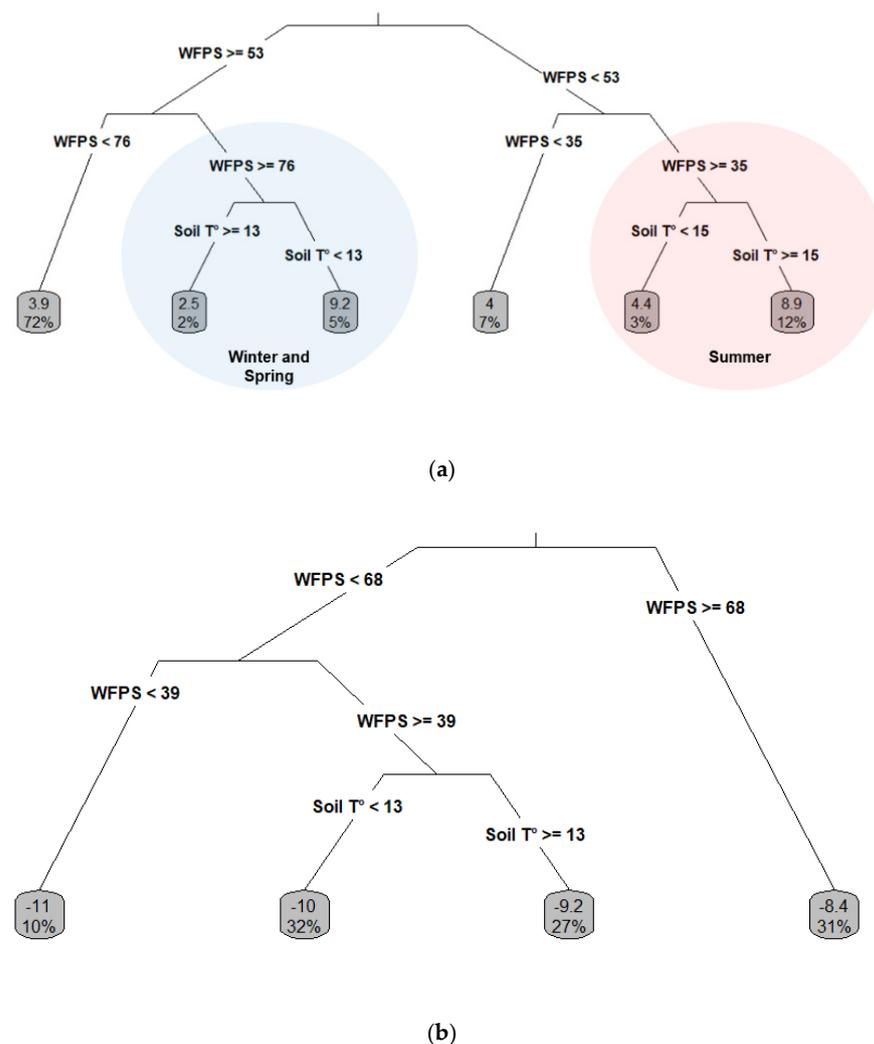


Figure 4. Key variables driving soil GHG fluxes in a temperate pasture, using tree decision analysis. (a) N-N₂O ($\text{g N-N}_2\text{O ha}^{-1} \text{ d}^{-1}$), and (b) C-CH₄ ($\text{g C-CH}_4 \text{ ha}^{-1} \text{ d}^{-1}$).

4. Discussion

4.1. Key Variables Driving The N-N₂O and C-CH₄ Fluxes

Fluxes of N-N₂O were mainly determined by WFPS and soil temperature, so that when WFPS was higher than 76% but not completely anaerobic, N-N₂O fluxes increased as soil temperature decreased under 13 °C. These are the typical climate conditions in winter–spring in Southern Chile when high N-N₂O emissions are expected due to high soil moisture content, despite low temperature, in agreement with Wang et al. [34]. These authors found that N-N₂O is rapidly produced when soil WFPS is increased over 60%, given that soil pore water reduces soil O₂ availability, increasing the anaerobic soil conditions and, therefore, the denitrification process. During the soil N cycle, NO₃⁻ is reduced to N-N₂O by denitrifying bacteria, and therefore, under these conditions, the increase in the soil NO₃⁻ will increase the N-N₂O production [34].

However, when WFPS was found to be below 53%, N-N₂O emissions increased as soil temperature increased over 15 °C, reflecting the typical climate and soil conditions on the summer season. This suggests that high N-N₂O emissions during summer are expected, even at low soil WFPS, in response to higher soil temperatures, which positively increase the activity of soil microbial communities (i.e., activity of the nitrifying and denitrifying bacteria) and biological oxygen consumption, resulting in anaerobic areas in the soil [35,36].

Similar results have been reported for temperate conditions in ref. [37,38], with N₂O fluxes mainly being determined by soil temperature, followed by other variables, such as soil NH₄ availability and WFPS. However, for tropical conditions, such as those presented for Brazil by da Silva Cardoso et al. [38], large N₂O fluxes were registered with soil temperature over 22 °C, while the Argentinian results by Cosentino et al. [37] showed large N₂O fluxes with temperature over 14 °C, which reflect the impact of geographical conditions (country) on N₂O emissions.

Fluxes of C-CH₄ were negative across the experiment, suggesting C capture in volcanic soils. In this case, WFPS reflects the role of soil moisture on stimulating CH₄ capture [39–41].

According to the tree decision analysis, when the soil WFPS was higher than 39%, C-CH₄ capture was reduced as the soil temperature increased over 13 °C, suggesting that the combination of high temperature and high soil moisture stimulate the CH₄ capture, in agreement with previous results of Mazzetto et al. [42]. Additionally, according to the review by Dalal et al. [39], when anaerobic conditions are maintained in the soil (typically after rain events or high WFPS), CH₄ capture increases with increasing temperatures, supporting our results. Thus, when WFPS was higher than 39% and soil temperature was below 13 °C, the C-CH₄ capture was increased, probably due to lack of soil conditions in terms of temperature. However, when the WFPS was lower than 39%, we observed the higher C-CH₄ capture rates, suggesting that soil moisture is more relevant to CH₄ capture than soil temperature. Similar results have been reported in ref. [38,42], where the WFPS was the main variable influencing CH₄ fluxes, followed by soil temperature.

4.2. Effects of Pasture Treatment on Methane and Nitrous Oxide Emissions

Emissions of N-N₂O and C-CH₄ were similar between treatments during the first experimental year. The emissions of N-N₂O were higher during spring, after treatment establishment, given that all treatments received the same N input and that soil tillage and seeding promote mineralization in the soil [43], resulting in higher soil availability of N and contributing to increased N₂O emissions. On the other hand, and in agreement with Dunfield [44], the soil C-CH₄ sink is affected by environmental condition, such as temperature and soil WFPS, due to their effect on gas diffusion into the soil. Additionally, high changes in WFPS can also affect the soil microbiology, probably resulting in the lower capture of C-CH₄ observed during autumn, where rainfall events are more frequent increasing the soil water content, reducing the diffusion of C-CH₄ into the soil in addition to causing an imbalance in the soil microbiology.

During the second year, there was no effect of treatments over cumulative fluxes of N-N₂O and C-CH₄. Even in treatments with legumes incorporation that did not

receive N fertilizer. Thus, biologically fixed N had a similar impact on emissions as that applied as fertilizer, in agreement with results by Shah [45]. This author explained that the decomposition and releasing of rapid C and N in nodules can be a source that increases N₂O emissions later on in time.

A seasonal effect was observed on N–N₂O and C–CH₄ emissions, with lower N–N₂O emissions being measured in winter when low temperature and high soil WFPS disfavors the soil microbiological processes, while during summer, N–N₂O emissions were increased and a major capture of C–CH₄ was observed, due to the beneficial environmental conditions for soil microbiological activity, with rainfall events and higher temperatures, resulting also in adequate optimal soil WFPS conditions for the diffusion of these gases.

A lower EI was observed in the control treatment during the first year, probably due to the initial high yield of the forage mix chosen by the farmer, compared to the perennial varieties proposed, which had a slower establishment in the first year, increasing its yield during the second year and thereafter, with the Br/L mixture having the lowest EI in time (see ahead). Additionally, this yield pattern affects farmers' economic return in the short term. This may represent a barrier for farmers' adoption, unless adequate knowledge transfer and extension is provided together with the technology.

4.3. Effects of Pasture Treatments on Pasture Yield and Chemical Composition (N Uptake)

The control showed a greater average pasture yield (first and second years), compared to other treatments, with the greatest ME and least CP content. A high pasture production with high energy content is required for dairy cattle systems based on pasture, given that energy is the main factor limiting milk production in grazing systems of Southern Chile [46]. Additionally, it has been observed that one strategy to improve N use efficiency and decrease urinary N excretion from grazing dairy cows is reducing the pasture N concentration and increasing the respective energy content, suggesting that the control treatment will supply the best combination of quantity and quality of the pasture to improve animal production and reduce N excretion. Recently, Beltran et al. [47] showed that greater N use efficiency for milk production (proportion of N intake which is retained in milk) and lower urinary N excretion were related to the non-fiber carbohydrates (NFC)/CP ratio of diet and NFC intake, respectively. The NFC content of the pasture is a source of energy quickly available in the rumen, supporting the importance of pasture energy to improve N retention in milk and thereby reducing environmental pollution related to non-CO₂ emissions, such as N₂O and NH₃ from urine and dung during grazing.

The control treatment showed 46–67% greater pasture yield than other treatments in the first year, given that this treatment was composed by annual grass species, which are characterized for a high pasture yield during the first year, compared to other treatments composed by perennial species, where the peak of pasture yield is reached in the second to third years [48].

Pasture species adapted to high temperature and low water availability will be required for future agriculture, when climate change will increase the duration of extreme high temperature events and heat stresses, reducing plants and animals' productivity in many regions [12]. In this study, we evaluated two prairie species adapted to high temperature and low water availability (*B. valdivianus*, and *L. corniculatus*) given by their deep and well-structured root systems (data not shown, example provided in Figure S1), both lone and mixed. The mix pasture (Br/L) produced the greater pasture yield during the second year of the study, mainly related to the reduction in yield in the control treatment. However, they showed an unbalanced chemical composition in terms of high CP and low energy content, probably requiring the use of low CP and high energy supplements to maintain the animal performance and reduce urinary N excretion [47], which is a relevant N₂O and NH₃ source from grazing systems [49,50].

Lotus is a legume species characterized for a high condensed tannins content [51], which can form complexes with protein by hydrogen bonds, protecting the dietary protein against rumen degradation, and consequently altering N excretion by reducing urinary N

excretion [52]. Nitrogen is mainly excreted by urine in ruminants, which is more readily available than N feces, being quickly volatilized as NH_3 or N_2O . Therefore, Lotus inclusion into the pasture mix could contribute to reducing the N_2O emissions and NH_3 volatilization in grazing systems.

The inclusion of Bromus in pastures has shown contrasting results. Turner et al. [53] reported that the use of *Bromus willdenowii* increased the pasture yield compared to *L. perenne* under dry conditions. However, López et al. [16] found similar pasture yields between a monoculture of *Bromus valdivianus* and *L. perenne* under different water restrictions. These results indicate that Bromus (subspecies valdivianus and willdenowii) may have different yields. In our study, the mix pasture of *B. valdivianus* and *L. corniculatus* improved the average pasture yield during the second year, possibly related to the extension of pasture growing imposed by complementary growth patterns between species, compared to the monoculture.

Therefore, the use of a mix pasture, such as *B. valdivianus* and *L. corniculatus* (Br/L treatment in this study), can be used to increase yield at critical dry periods during the year, contributing as well to GHG mitigation; however, low CP and high energy supplements should be considered, to reduce the risk of high N excretion to the wider environment.

5. Conclusions

Overall, the results of the current study revealed that a mix pasture of *B. valdivianus* and *L. corniculatus* can be used as a farming strategy to favor pasture yield at critical dry periods during the year. This approach will provide greater yield after year one of establishment, although low CP and high energy supplements should be considered for animal feed, to reduce the risk of high N excretion to the wider environment by grazing animals. This management also reduces N– N_2O emissions from grassland soils as well as favoring C– CH_4 capture. Our study determined that N– N_2O and C– CH_4 emissions were determined by soil variables, mainly WFPS, and secondarily by soil temperature. Additionally, this pasture mix required a lower N fertilization, resulting in a reduction in the pasture production cost. Extension and knowledge transfer should be provided to farmers to account for potential adoption barriers, such as low short-term yield.

Supplementary Materials: The following are available online at <https://www.mdpi.com/article/10.3390/agronomy12051097/s1>, Figure S1: Examples of root development for all treatments (0–0.8 m depth), Table S1: Dates and rates of nutrients application to the different treatments through the experimental period, Table S2: Example of the variation in the N– N_2O daily flux ($\mu\text{g N}_2\text{O-N m}^{-2} \text{hr}^{-1}$) in three chambers at three different dates.

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