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Article

In-Vitro Fermentation of Browsable Native Shrubs in New Zealand

Chege J. Wangui *, James P. Millner, Paul R. Kenyon, Peter R. Tozer, Patrick C H,Morel and Sarah J. Pain

School of Agriculture and Environment, Massey University, Private Bag 11 222, Palmerston North 4442, New Zealand; J.P.Millner@massey.ac.nz (J.P.M); P.R.Kenyon@massey.ac.nz (P.R.K.); P.Tozer@massey.ac.nz (P.R.T.); (P.C.H.M) P.C.Morel@massey.ac.nz; S.J.Pain@massey.ac.nz (S.J.P)

* Correspondence: J.Wangui@massey.ac.nz

Abstract: Information on the nutritive value and *in vitro* fermentation characteristics of native shrubs in New Zealand is scant. This is despite their potential as alternatives to exotic trees and shrubs for supplementary fodder, and mitigation of greenhouse gas and soil erosion on hill country sheep and beef farms. The objectives of this study were to measure the *in vitro* fermentation gas production, predict parameters of *in vitro* fermentation kinetics and to estimate *in vitro* fermentation of volatile fatty acids (VFA), microbial biomass (MBM) and greenhouse gases of four native shrubs (*Coprosma robusta*, *Griselinia littoralis*, *Hoheria populnea* and *Pittosporum crassifolium*) and an exotic fodder tree species, *Salix schwerinii*. Total *in vitro* gas production was higher ($p<0.05$) for natives than *S. schwerinii*. Prediction using the single pool model resulted in biologically incorrect negative *in vitro* total gas production from the immediately soluble fraction of the native shrubs. However, the dual pool model better predicted *in vitro* total gas production and was in alignment with measured *in vitro* fermentation end products. *In vitro* VFA and greenhouse gas production from fermentation of leaf and stem material were higher ($p<0.05$), and MBM lower ($p<0.05$), for native shrubs compared to *S. schwerinii*. The lower *in vitro* total gas production, VFA and greenhouse gases production, and higher MBM of *S. schwerinii* may be explained by the presence of condensed tannins (CT), although this was not measured and requires further study. In conclusion, results from this study suggests that when consumed by ruminant livestock, the browsable native shrubs can provide adequate energy and microbial protein, and that greenhouse gas production from these species is within ranges reported for typical New Zealand pastures.

Keywords: Native shrubs; In vitro fermentation; volatile fatty acids; greenhouse gases; hill country

1. Introduction

Sheep are efficient utilizers of pastures due to the symbiotic anaerobic microbiota, mainly bacteria, fungi and protozoa in their reticulorumen [1–3]. These microorganisms obtain nutrients by fermentatively breaking down the ingested feed, in return providing organic acids, microbial proteins, and some B complex vitamins to the host [4]. These fermentation processes also produce gases, primarily carbon dioxide and methane, which are major greenhouse gases [4]. Organic acids, principally acetic, propionic, and butyric volatile fatty acids (VFA) supply 70 to 80% of the dietary energy [4] of ruminants, while microbial protein provides 70 to 100% of the amino acids required by ruminants [5]. However, diet influences the reticulorumen microbial profile, which determines the rate of substrate fermentation and VFA and gas composition [4,6,7]. Fibrous diets are slow to degrade and encourage acetogenic anaerobes and methane gas production, while diets rich in simple carbohydrates are highly fermentable and promote mainly propiogenic microbes and carbon dioxide gas production [6,7].

Both *in vivo* and *in vitro* methods can be used to evaluate feed substrate digestibility and their nutritive value, but *in vitro* methods are preferred because they are a convenient and inexpensive proxy for *in-vivo* fermentation [3,8]. *In vitro* methods involve incubation of feed substrate in buffered rumen fluid with measurement of gas production periodically, and VFA and residue at the end of the fermentation process [4,9]. Unlike *in vivo*

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methods where VFA are absorbed across the rumen wall, VFA accumulate in rumen fluids *in vitro* and their concentration indicates the actual production from the substrate [4]. Similar to *in vivo* methods, the residue obtained after the fermentation process can be used to determine the substrate digestibility and the microbial protein utilizable by the host [7].

Gas produced *in vitro* correlates with the rate of substrate fermentation and can be fitted to mathematical functions to estimate the feed substrate fermentation kinetic parameters [3,10,11]. Non-linear mathematical functions, mainly exponential and sigmoidal, are preferred because of their ability to model microbial growth and have parameters that can be biologically explained [11–13]. However, the suitability of the mathematical function in estimating fermentation kinetic parameters depends on the nutritive composition of the feed substrate [3,13,14]. Single compartment models, also referred to as single pool functions assume a constant rate of fermentation and are mainly suited for feed substrates with homogenous nutrients, while dual pool functions considers two rates of fermentation and are suitable for feed substrates with heterogenous nutrients [11–14]. Application of both single and dual pool non-linear functions is common in feed evaluation because natural feeds consumed by sheep contain a mixture of substrates that vary in nutrient composition [11].

Pasture, predominantly perennial ryegrass with a small proportion of clover, is the cheapest and most widely utilized feed resource in hill country sheep and beef farms in New Zealand [15–17]. In addition, supplementary feeds are used during seasons of low pasture supply and quality [17,18]. Potential supplementary feeds used in the hill country sheep and beef farms can include conserved forages, alternative grazed forages, and concentrates [18]. Further, foliage harvested from poplar and willow trees, commonly used for soil conservation in the hill country sheep and beef farms can be used as sources of supplementary forage during the summer season [19–21]. Native shrubs may also offer potential feed source when browsed *in situ* [22–25], but this has not yet been widely explored [22,23].

Native shrubs are merited for cultural value, genetic diversity, adaptability, soil conservation, and they are evergreen and can supply foliage all year-round [23,26]. Some native shrubs species are highly preferred by wild herbivores suggesting their potential as a livestock feed resource [24,25,27]. However, their nutritive value, *in vitro* fermentation gas production, fermentation kinetics and fermentation end products in domesticated ruminants have not been previously studied. Information on the nutritive value, *in vitro* fermentation gas production, fermentation kinetics and fermentation end products of New Zealand native shrubs could be used for comparison with conventional feed resources, which would aid in decision making by policy makers, researchers, and hill country farmers. The objective of this study was therefore, to determine the nutritive value, *in vitro* fermentation gas production, fermentation kinetics and fermentation end products of four New Zealand native shrubs with potential use as sheep fodder in comparison to an exotic shrub utilized in the North Island hill country sheep and beef farms.

2. Materials and MethodsShrubs and study site description

Four native shrub species, *Coprosma robusta* (Karamū), *Griselinia littoralis* (Pāpāuma), *Hoheria populnea* (Houhere) and *Pittosporum crassifolium* (Karo), and an exotic osier willow, *Salix schwerinii* (Kinuyanagi) were compared in this study. The five selected species were among eight shrubs planted in August 2019 on a fenced trial site located (Lat -40.401447, Long 175.617912) at the Massey University No 4 Dairy farm, five kilometers south of Palmerston North. Other shrubs present but not included in the comparison were *Pseudopanax arboreus* and Hawke's Bay ecotypes of *G. littoralis* and *P. arboreus*. They were not included due to insufficient herbage production.

The trial site was set up in a randomized complete block design with four blocks. Each block had eight plots randomly allocated to shrub species or ecotype. Plots were planted with 15 shrubs in three rows and five columns and spaced at 1.5m by 1.5m. The trial site was on a southerly aspect steep slope (>25°) dominated by Tokomaru silt loam soil at the top and Ohakea silt at the bottom [28]. Tokomaru silt loam and Ohakea silt loam

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are characterized as having average natural fertility and fair to poor drainage and are mainly used for pastoral farming [28]. Prior to planting, the site was used for dairy cattle grazing. The climatic conditions for Palmerston North are defined in Table 1 [29].

Table 1. Summarized long-term climatic conditions for Palmerston North district (Source. [29,30])

Climate parameters		Season			
		Summer	Autumn	Winter	Spring
Temperature	Mean (°C)	17.4	13.8	9.0	12.4
Rainfall	Total (mm)	222.0	189.0	246.0	43.7
	Percent of total rainfall (%)	25.0	21.0	27.0	26.0
Wind	Mean speed (km/h)	15.8	14.0	13.9	16.8
Solar radiation	Mean daily (MJ/m2/d)	21.1	11.0	6.7	15.9

2.2. Sample collection and processing

Foliage samples, consisting of leaved stems with a diameter of less than 5 mm, were collected in October 2020. Five shrubs in each plot were randomly selected and at least five foliage samples harvested from each shrub. A total of 20 foliage samples were collected comprising of five shrub species in each of the four blocks. The foliage samples were indiscriminately harvested from the lower, middle, and top parts (not higher than 1.1m) of the shrub to imitate the browsing behavior of sheep and to ensure the samples were representative of the entire shrub. Foliage samples for each shrub species from each plot were pooled, labeled, and chilled to approximately 4°C while being processed. Any foreign materials such as grass, dead leaves and spider webs were removed from the collected samples (n=20, i.e., five species by four pooled samples), before further separating the foliage samples into leaves (n=20) and stems (n=20) sub-samples. The leaf sub-sample included the leaf blade, stipules, buds, and petiole. The stem sub-sample included the woody and soft bark where the leaves were attached. The sub-samples were frozen before submission to the Massey University Food and Nutrition Laboratory for freeze drying, grinding and proximate analysis of nutrients. A portion of the ground sub-samples (20 leaf and 20 stems; n=40) was submitted to Alltech laboratories, Auckland for *in vitro* fermentation analysis.

2.3. Proximate analysis and *in vitro* digestibility

The foliage sub-sample dry matter content (DM) was determined as percentage of the weight remaining after moisture loss during freeze drying and was estimated using the AOAC 925.10, 930.15 calculation. Pyrolysis and combustion following AOAC 968.06 (Dumas) method was used to estimate total nitrogen, which was multiplied by 6.25 to estimate the crude protein (CP) content in the sub-samples. Ash content was determined by combusting the organic matter (OM) portion of the DM in a Furnace at 550°C following the AOAC 942.05 (Feed, meat) method. Fibre fractions were estimated following AOAC 2002.04 method for neutral detergent fibre (NDF) and AOAC 973.18 for acid detergent fibre (ADF) and acid detergent lignin (ADL) using the Fibretec system.

In vitro dry matter digestibility (IVDMD) and organic matter digestibility (IVOMD) were estimated by treating samples with a neutral detergent solution and digesting with pepsin and fungal cellulase enzymes as described by [31] and were expressed as percentage of the DM. Digestible organic content in the dry matter (IVDOMD) was calculated as a product of sub-sample OM (100- ash) and IVOMD and expressed as a percentage of the DM. The IVDOMD was used to derive the metabolizable energy (ME) in megajoules per kilogram of DM (MJ/kg DM) by multiplying by a factor of 0.16 [32].

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2.4. Measuring *in vitro* fermentation gas production

In vitro fermentation was carried out using the Alltech IFM™ tool to determine fermentation gas production, VFA and microbial biomass (MBM). The procedure used is described by [33]. Each sub-sample (20 leaf and 20 stems; n=40) was duplicated into 0.5g portions to be incubated in an *in vitro* medium made up of a mixture of rumen fluid inoculum and buffer solution. Rumen fluid was collected from a fistulated lactating dairy cow in the morning approximately two hours after *ad lib* feeding on pasture and supplemented with grass and maize silage, 0.5kg of molasses, and 1.5kg pelleted dairy concentrate. The freshly collected rumen fluid was filtered through a double layer of cheese cloth to remove undigested material and was mixed with 5.6 L of McDougall bicarbonate buffer solution [34] and 250 ml of a reducing agent to make an *in vitro* medium with a 20:80 rumen fluid to buffer ratio. Each sub-sample portion was added into 100ml of *in vitro* medium in 250ml bottles and incubated at 39°C for 48 hours with gentle stirring and periodic pH checks [35]. Fermentation gas production was measured using an automated pressure transducer and recorded continuously for 48 hours [36]. The recorded gas production was used to estimate the *in-vitro* fermentation kinetics for the shrub species.

2.5. Estimating the *in vitro* fermentation kinetics parameters

Nonlinear mathematical models are widely used to fit *in vitro* gas production to describe the fermentation kinetics of ruminant feeds [14]. The nonlinear models vary in structure, parameters, time zero behavior, lag period, points of inflection, and fermentation gas pools [3,14]. Description of some nonlinear models commonly used to fit *in vitro* gas production can be found in [3,11,13,14]. However, the exponential and sigmoidal models are preferred because they are robust in relating gas production to microbial mass and substrate levels [11] and can be structured to accommodate more than one fermentation gas pool [14]. Single pool models are better predictors of fermentation kinetics of simple substrates and are suggested for feeds with low fibre, while multipool models are better descriptor of heterogenous substrate and considers each substrate fraction independently and are recommended for feeds with high fibre [14]. Combined use of single and multipool models has been suggested when determining the best model that describes fermentation kinetics where substrate fractions in the test feeds are unknown [14]. Exponential single pool [37] (equation 1) and logistic dual pool [11] (equation 2) models are extensively used and validated in fitting *in vitro* fermentation gas production [13,38] and were selected to describe the *in vitro* fermentation kinetics of the study shrub species.

Measured gas production for each sub-sample was combined for each shrub species and fitted using SAS non-linear procedure (Proc NLIN) to estimate model parameters that describe the rate and volumes of gas production from the sub-samples at given time (t) in hours. In the single pool model, total gas production (V_{ors}) in milliliters per gram of dry matter (mL/g DM) was the sum of gas production from the highly (a, mL/g DM) and slowly (b, mL/g DM) fermentable nutrients, produced at similar fermentation rate (c) expressed in percentage gas production per hour (%/h). Total gas production in the dual pool model, $(V_{Sch}, \text{mL/g DM})$ was the sum of gas production from the fast pool (V_1, mL/g DM) portion and the slow pool (V_2, mL/g DM) produced from the highly fermentable and slowly fermentable substrate fractions, respectively. The V_1 and V_2 were assumed to have similar lag (L) time, but the rates of gas production (C_1, %/h) for V_1 and (C_2, %/h) for V_2 were different.

$$V_{Ors} = a + b(1 - e^{-ct}) \quad (1)$$

$$V_{Sch} = \left[\left(V_1/\left(1 + e^{(2+4\times C_1(L-t))}\right)\right) + \left(V_2/\left(1 + e^{(2+4\times C_2(L-t))}\right)\right)\right] \quad (2)$$

Shrub total gas production after 24 hours for the single pool (V_{24}, mL/g DM) and dual pool ($V_{1_{24}}$ and $V_{2_{24}}$, mL/g DM) was determined at t= 24 h for both models. The gas production half-life was assumed to be time (t, h) at which, half of the total gas for each pool was produced [13]. Gas production half-life was estimated using the t function of the [37] ($T_{0.5}$, h) (equation 3) and [11] ($V_{T_{0.5}}$, h) (equation 4) models.

$$T_{0.5} = -\left(\ln\left(-{}^{0.5V_{Ors}}/_{b}\right) + \left({}^{a}/_{b}\right) + 1\right)/c \quad (3)$$

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$$V_{T_{0.5}} = -(\ln(-0.5)/4c) + L + (1/2c), \quad (4)$$

Where, $V_{T_{0.5}}$ was $V_{1_{T_{0.5}}}$ or $V_{2_{T_{0.5}}}$

Model performance was determined by regressing predicted (x-axis) against the observed (y-axis) [13,39–41] gas production over 48 h. Regression residuals were used to determine the model's goodness of fit and accuracy using root mean square error (RMSE) and mean absolute percentage error (MAPE) metrics [42,43]. Since RMSE units were similar to the regressed variables, low RMSE indicated high fitness of fit of the models [43]. A MAPE less than 5% showed excellent, 10 to 25% good and greater than 25% very low and unacceptable model prediction accuracy [42]. Adjusted coefficient of determination (adjusted R^2) was used to indicate the proportion of variability explained by the models, with values close to one suggesting a stronger relationship between the predicted and observed gas production values [43].

2.6. *In vitro* fermentation end products

After 48 h of *in vitro* fermentation the medium (section 2.4) pH was measured before centrifuging. The supernatant was used to determine the VFA concentration, and the residues were used to estimate the sub-sample's digestibility and the MBM. Volatile fatty acids (acetate, propionate, butyrate and valerate) and their isomers (isobutyrate and isovalerate) were recovered using the method suggested by [44] and their concentration determined using the Agilent GC 7890 (Flame Ionization Detector, FID) gas chromatography system. Total VFA were expressed in millimolar (mM) concentration while individual VFA and their isomers were expressed as the percentage of the total VFA. The concentration of individual VFA was used to balance theoretical fermentation equations to predict the volume (mL) and mass (g) of carbon dioxide (CO_2) and methane (CH_4) gases produced per gram of DM (g/DM) during the sub-samples fermentation [45]. Predicted fermentation gases proportions were pooled using their global warming potential [46] to determine the CO_2 equivalent (Eq CO_2) emission potential of the shrub samples. The residue (undegraded sub-samples containing MBM) weight was used to determine the apparent DM digestibility (aDMD) [47] while the weight after solubilization of the MBM was used to estimate the true DM digestibility (tDMD) [48] as a percentage of the DM. The MBM (mg/g DM) yield was estimated as the difference between aDMD and tDMD weights [48].

2.7. Statistical analysis

SAS software version 9.4 was used to carry out the statistical analysis. Analysis of variance in the general linear model procedure (proc GLM) was used to compare the difference among the shrub samples means for the leaves and stems for the proximate nutrients and *in vitro* fermentation end products. The means were considered different if p> 0.05 and were separated using the Tukey method.

3. Results

3.1. Shrubs nutritional composition

The proximate nutritional composition of both the leaves and stems differed ($p<0.05$) between shrub species, except for NDF content in the leaves ($p>0.05$) (Table 2). Leaf DM was similar ($p>0.05$) in both *P. crassifolium* and *S. schwerinii*, which were higher ($p<0.05$) than all other species. Ash content was higher ($p<0.05$) in *H. populnea* and lower ($p<0.05$) in *S. schwerinii* leaves, than in all the other species. The CP was similar ($p>0.05$) in *H. populnea* and *S. schwerinii* leaves, and higher ($p<0.05$) than all other species, which did not differ ($p>0.05$). *Salix schwerinii* leaves had higher ($p<0.05$) ADF than *H. populnea* and *P. crassifolium*, which were similar ($p>0.05$), while *C. robusta* and *G. littoralis* did not differ ($p>0.05$) from the other species. Lignin was higher ($p<0.05$) in *G. littoralis* and lower ($p<0.05$) in *S. schwerinii* and *H. populnea*, while *C. robusta* and *P. crassifolium* were intermediate and did not differ ($p>0.05$) from the other species. The ME for *C. robusta, G. littoralis* and *P.*

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crassifolium leaves was similar ($p>0.05$) and higher ($p<0.05$) than the remaining species, which did not differ ($p>0.05$).

Table 2. Native (*Coprosma robusta, Griselinia littoralis, Hoheria populnea* and *Pittosporum crassifolium*) and exotic (*Salix schwerinii*) shrub species leaf (n=20) and stem (n=20) dry matter (DM, %) as a percentage of the fresh weight, and ash (Ash, %), crude protein (CP, %), neutral detergent fibre (NDF, %), acid detergent fibre (ADF, %) and lignin (Lignin, %) as percentage of the DM and metabolizable energy (ME, MJ/kg DM).

Shrub species	DM	Ash	CP	NDF	ADF	Lignin	ME
			Leaves				
Coprosma robusta	39.3^{b}	7.0^{bc}	7.9^{b}	37.4	22.4^{ab}	9.6^{ab}	12.0^{a}
Griselinia littoralis	31.5^{c}	7.3^{b}	6.2^{b}	32.1	22.3^{ab}	12.0^{a}	11.9^{a}
Hoheria populnea	31.1^{c}	11.6^{a}	14.0^{a}	37.6	20.2^{b}	7.5^{b}	11.4^{b}
Pittosporum crassifolium	43.0^{a}	6.5^{c}	6.2^{b}	36.4	20.7^{b}	9.3^{ab}	12.0^{a}
Salix schwerinii	43.1^{a}	4.9^{d}	15.1^{a}	36.3	24.6^{a}	8.8^{b}	11.3^{b}
Pooled SE	0.57	0.17	0.41	1.31	0.63	0.62	0.07
			Stems				
Coprosma robusta	35.8^{b}	6.4^{bc}	4.7^{b}	46.6^{c}	36.7^{b}	9.1^{b}	10.2^{a}
Griselinia littoralis	32.1^{b}	5.6^{c}	4.2^{b}	48.6^{bc}	38.6^{ab}	10.2^{ab}	10.0^{ab}
Hoheria populnea	36.0^{b}	9.0^{a}	8.3^{a}	53.5^{a}	41.5^{a}	9.2^{b}	9.6^{b}
Pittosporum crassifolium	47.4^{a}	6.7^{b}	4.2^{b}	52.0^{ab}	41.0^{ab}	8.8^{b}	9.6^{b}
Salix schwerinii	48.5^{a}	3.7^{d}	7.0^{a}	48.9^{bc}	38.4^{ab}	11.6^{a}	10.2^{a}
Pooled SE	1.05	0.23	0.37	0.95	1.07	0.38	0.13

Nutrients value with different superscripts in a column for the sample type are different at p< 0.05

Salix schwerinii and *P. crassifolium* had similar ($p>0.05$) stem DM, which was higher ($p<0.05$) than the other species. *Hoheria populnea* had higher ($p<0.05$) and *S. schwerinii* lower ($p<0.05$) stem ash, than all other species. Stem CP was similar ($p>0.05$) in *H. populnea* and *S. schwerinii*, and higher ($p<0.05$) than the other species, which were not different ($p>0.05$). Stem ADF and NDF were higher ($p<0.05$) in *H. populnea* and lower ($p<0.05$) in C. *robusta*, compared to the other species. Unlike in the leaves, *S. schwerinii* had higher ($p<0.05$) stem lignin content than all other species, except *G. littoralis*, which was comparable ($p>0.05$) to the other species. Stem ME was similar ($p>0.05$) in C. *robusta* and *S. schwerinii,* and higher ($p<0.05$) than the other species, except for *G. littoralis*, which had intermediate stem ME.

3.2. *In vitro* fermentation gas production and kinetics

3.2.1. *In vitro gas production*

The total gas production from *in vitro* fermentation for the leaves and stems differed ($p<0.05$) among species (Table 3). For both leaf and stem material, *S. schwerinii* had lower ($p<0.05$) gas production compared to the native shrub species. Gas production from leaves was similar ($p>0.05$) to that of stem for *H. populnea* and *S. schwerinii*, but for other species, gas production was higher ($p<0.05$) for leaves than for stems.

Table 3. Total gas production in millilitres per gram of dry matter (mL/ gDM) from the *in vitro* fermentation of leaf (n=20) and stem (n=20) material from native (*Coprosma robusta, Griselinia littoralis, Hoheria populnea* and *Pittosporum crassifolium*) and exotic (*Salix schwerinii*) shrub species.

Species	Leaves	Stems	SE
Coprosma robusta	157.0^{a}	105.9^{a†}	6.92

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Griselinia littoralis	151.7^{ab}	105.9^{a†}	5.01
Hoheria populnea	116.6^{b}	108.6^{a}	7.01
Pittosporum crassifolium	135.3^{ab}	100.8^{a†}	8.80
Salix schwerinii	46.1^{c}	68.6^{b}	6.58
SE	8.42	5.12	

^{abc} *Mean total gas production for the shrubs in a column with different letter superscript differ significantly at p<0.05.*

† *Mean total gas production between leaves and stems in a row with symbol superscript differ significantly at p<0.05.*

3.2.2. Shrubs in vitro fermentation kinetics

Parameter estimates of *in vitro* fermentation kinetics for the shrub leaves using the single pool model are shown in Table 4 and resulting gas production curves in Figure 1(a). The function parameters for the immediately soluble fraction (*a*, ml/g DM) were negative for the native shrubs. Gas production from the slowly degradable fraction (*b*, ml/g DM) and total gas production (V_{ors}, ml/ gDM)) were more than three times higher in the native shrubs than in *S. schwerinii*. However, *H. populnea* had a slower rate of gas production (*c*, %/h) than all other species resulting in lower gas production after 24 hours (V_{24}, ml/ gDM) and the longest gas production half-life ($T_{0.5}$, h). Native shrub species had better model performance for the leaves compared to *S. schwerinii* which had very low accuracy (MAPE = 21.54) and a weak relationship (adjusted R^2= 0.414) between observed and predicted gas production.

Parameter estimates for stem *in vitro* fermentation kinetics (Table 5 and Figure 1(b)) were negative, except in *C. robusta* and *S. schwerinii*. The *b* and V_{ors} parameters were higher in the natives than in *S. schwerinii*. However, *C. robusta* and *S. schwerinii* had lower *c* resulting in lower V_{24} compared to other shrub species. In contrast to the leaf model, the model for *S. schwerinii* stems had the best fit (RMSE= 4.25) and explained a larger proportion of variability (adjusted R^2= 0.935) between the predicted and observed gas production compared to the native shrub species and had a high mean absolute percentage error (MAPE = 3.14).

Table 4. Native (*Coprosma robusta, Griselinia littoralis, Hoheria populnea* and *Pittosporum crassifolium*) and exotic (*Salix schwerinii*) shrub species leaf *in vitro* fermentation kinetic parameters derived using single pool model. Where, *a*, gas production from the immediately soluble fraction (mL/g DM); *b*, gas production from the slowly degradable fraction (ml/g DM); *c*, rate of gas production (%/h); V_{Ors}, total gas production (ml/ gDM); V_{24}, total gas production after 24 hours (ml/ gDM), and $t_{0.5,}$ half-life of total gas production (h).

Species	Parameters	*a*	*b*	*c*	V_{ors}	V_{24}	$T_{0.5}$	MAPE	RMSE	Adj R^2
Coprosma robusta	Value	-1.0	165.3	0.070	164.3	133.1	10.2	-0.59	11.48	0.932
	SE	0.99	0.93	0.001						
	Low 95% CI limit	-3	163.5	0.067						
	Upper 95% CI limit	0.9	167.1	0.072						
Griselinia littoralis	Value	-6.9	166.6	0.079	159.7	134.5	9.9	2.18	5.69	0.983
	SE	0.51	0.47	0.001						

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	Low 95% CI limit	-7.9	165.7	0.078						
	Upper 95% CI limit	-5.9	167.5	0.08						
Hoheria populnea	Value	-3.5	139.7	0.043	136.2	85.9	17.5	-1.65	11.86	0.892
	SE	0.91	1.44	0.001						
	Low 95% CI limit	-5.3	136.8	0.040						
	Upper 95% CI limit	-1.7	142.5	0.045						
Pittosporum crassifolium	Value	-4.4	146.7	0.091	142.3	125.9	8.3	2.56	16.17	0.840
	SE	1.53	1.41	0.002						
	Low 95% CI limit	-7.4	143.9	0.087						
	Upper 95% CI limit	-1.4	149.5	0.096						
Salix schwerinii	Value	2.4	43.2	0.064	45.6	36.3	9.2	-21.54	13.25	0.414
	SE	1.12	1.08	0.005						
	Low 95% CI limit	0.2	41.1	0.054						
	Upper 95% CI limit	4.6	45.3	0.073						

RMSE, Root mean square error.

MAPE, Mean Absolute Percentage Error.

Adj R^2, Adjusted R squared.

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Table 5. Native (*Coprosma robusta, Griselinia littoralis, Hoheria populnea* and *Pittosporum crassifolium*) and exotic (*Salix schwerinii*) shrub species stem *in vitro* fermentation kinetic parameters derived using single pool model. Where, *a*, gas production from the immediately soluble fraction (mL/g DM); *b*, gas production from the slowly degradable fraction (ml/g DM); *c*, rate of gas production (%/h); V_{ors}, total gas production (ml/ gDM); V_{24}, total gas production after 24 hours (ml/ gDM), and $t_{0.5}$, half-life of total gas production (h).

Species	Parameters	a	b	c	V_{ors}	V_{24}	$T_{0.5}$	MAPE	RMSE	Adj R^2
Coprosma robusta	Value	4.9	104	0.055	108.9	81.2	11.0	-2.46	7.22	0.931
	SE	0.59	0.63	0.001						
	Low 95% CI limit	3.7	102.7	0.053						
	Upper 95% CI limit	6.0	105.2	0.057						
Griselinia littoralis	Value	-4.5	115	0.09	110.5	97.3	8.6	-0.07	10.94	0.876
	SE	1.03	0.95	0.002						
	Low 95% CI limit	-6.5	113.1	0.087						
	Upper 95% CI limit	-2.5	116.8	0.094						
Hoheria populnea	Value	-4.1	113.7	0.082	109.6	93.7	9.4	-0.18	11.01	0.875
	SE	1.0	0.92	0.002						
	Low 95% CI limit	-6.1	111.9	0.079						
	Upper 95% CI limit	-2.1	115.5	0.085						
Pittosporum crassifolium	Value	-2.0	100.1	0.14	98.1	94.7	5.2	0.16	6.78	0.920
	SE	0.75	0.73	0.002						
	Low 95% CI limit	-3.5	98.7	0.137						
	Upper 95% CI limit	-0.5	101.5	0.144						
Salix schwerinii	Value	5.6	62.9	0.057	68.5	52.4	9.3	3.14	4.25	0.935
	SE	0.35	0.36	0.001						
	Low 95% CI limit	4.9	62.2	0.055						
	Upper 95% CI limit	6.3	63.6	0.059						

RMSE, Root mean square error.

MAPE, Mean Absolute Percentage Error.

Adj R^2, Adjusted R squared.

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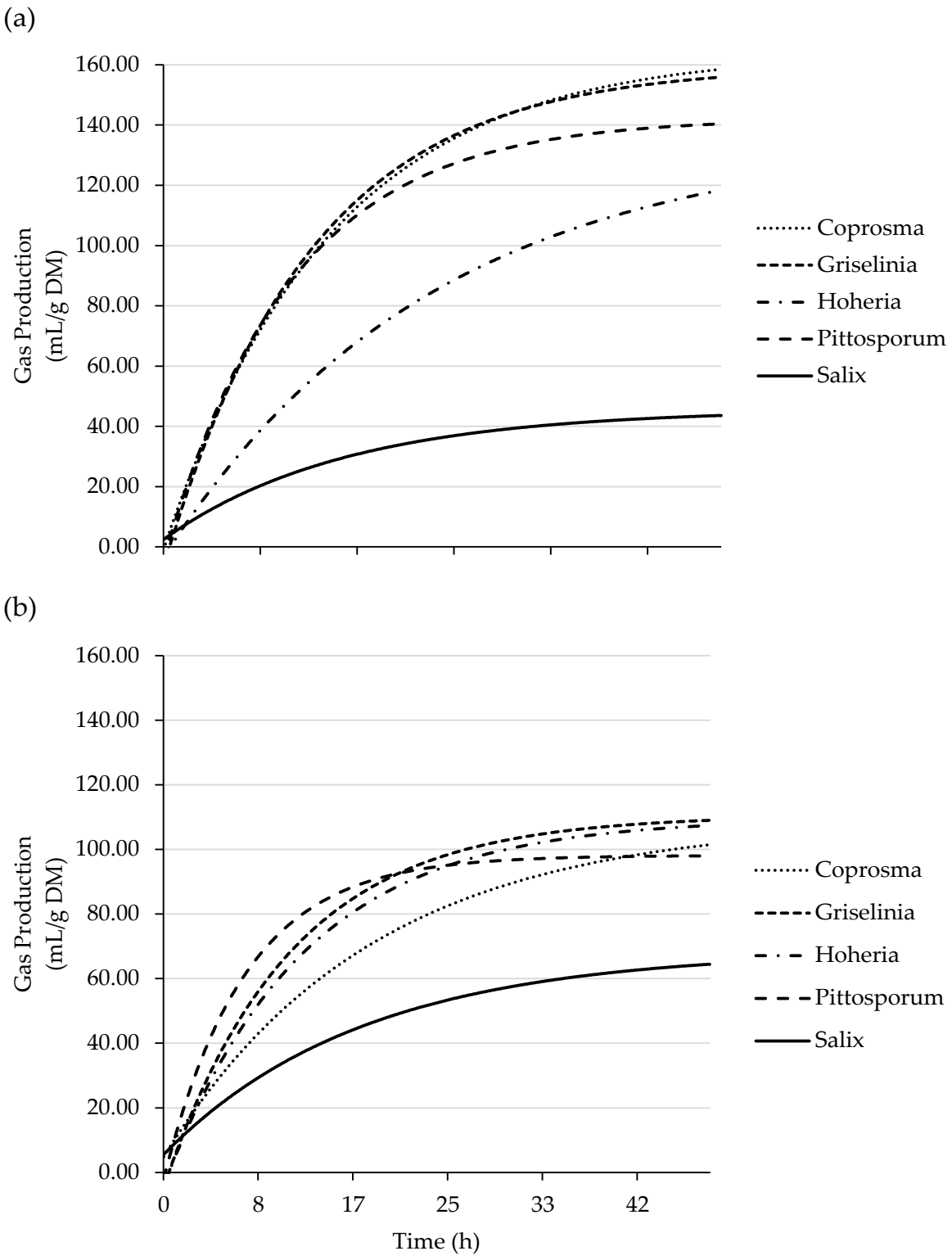

Figure 1. Predicted cumulative gas production curves over 48 hours for (a) leaves and (b) stems of native (*Coprosma robusta, Griselinia littoralis, Hoheria populnea* and *Pittosporum crassifolium*) and exotic (*Salix schwerinii*) shrub species using the single pool model.

The dual pool model *in vitro* fermentation kinetic parameter estimates for leaves and stems are shown in Tables 6 and 7 and the resulting predicted gas production curves in Figure 2 (a) and (b), respectively. Predicted leaf gas production from the fast pool (V_1, ml/g

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DM) was more than three times higher in the native shrub species compared to *S. schwerinii*. However, *S. schwerinii* had the highest rate of gas production for the fast pool (R_1, %/hr) resulting in a shorter gas production half-life ($V_{1_{T_{0.5}}}$, h) compared to the native shrub species. Lag time (L, h) was shortest in *S. schwerinii* and longest in *H. populnea*. Gas production from the slow pool (V_2, ml/g DM) was higher in the natives compared to *S. schwerinii*, except in *C. robusta*. Unlike in V_1, *S. schwerinii* had the lowest rate of gas production for the slow pool (R_2, %/hr) resulting in more than four times longer slow pool gas production half-life ($V_{2_{T_{0.5}}}$, h) compared to the native shrub species. Gas production after 24 hours ($V_{1_{24}}$ and $V_{2_{24}}$, ml/g DM) was more than three times higher in the native shrub species compared to *S. schwerinii*. Similarly, the performance of the dual pool model was better for the native shrub species compared to *S. schwerinii* which had very low accuracy (MAPE= 22.42) and the relationship between the observed and predicted gas production was weaker (adjusted R^2= 0.417) (Table 6).

Stems V_1 was lowest in *H. populnea* compared to the other species. *Salix schwerinii* had the lowest R_1 but shortest $V_{1_{T_{0.5}}}$ Stem L was highest for *C. robusta* followed by *S. schwerinii* and lowest for *P. crassifolium*. *Hoheria populnea* had the highest V_2 followed by *G. littoralis*, while the other species were similarly lower. *Pittosporum crassifolium* had the highest R_2 resulting in the shortest $V_{2_{T_{0.5}}}$ compared to *S. schwerinii* which had more than four times longer $V_{2_{T_{0.5}}}$. The dual pool model for *S. schwerinii* stems had the best fit (RMSE= 3.71) and explained a larger proportion of the variability (adjusted R^2= 0.950) between the predicted and observed gas production compared to the native shrub species and had a relatively good MAPE (-0.409) (Table 7).

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Table 6. Native (*Coprosma robusta, Griselinia littoralis, Hoheria populnea* and *Pittosporum crassifolium*) and exotic (*Salix schwerinii*) shrub species leaf *in vitro* fermentation kinetic parameters derived using dual pool model. Where, L, lag time (h); V_1, Fast pool total gas production (ml/g DM); V_2, Slow pool (ml/g DM); R_1, Fast pool rate of gas production (%/h); R_2, Slow rate (%/h), $V_{Sch,}$ total gas production, (ml/ g DM); $V_{1_{24}}$, total gas production for the fast pool after 24 hours (ml/g DM); $V_{2_{24}}$ slow pool after 24 hours (ml/g DM); $V_{1_{T_{0.5}}}$, fast pool total gas production half-life (h) and $V_{2_{T_{0.5}}}$, slow pool half-life (h)

Species	Parameters	V_1	R_1	L	V_2	R_2	V_{Sch}	$V_{1_{24}}$	$V_{1_{T_{0.5}}}$	$V_{2_{24}}$	$V_{2_{T_{0.5}}}$	MAPE	RMSE	Adj R^2
Coprosma robusta	Value	120.3	0.043	1.36	33.02	0.191	153.3	104.3	12.3	33.0	5.4	-8.29	11.32	0.934
	SE	1.86	0.001	0.18	1.94	0.019								
	Low CI	116.7	0.042	1.01	29.2	0.154								
	Upper 95% CI limit	124.0	0.044	1.71	36.8	0.228								
Griselinia littoralis	Value	92.2	0.040	1.84	60.8	0.123	153.0	75.8	13.6	60.8	6.9	-3.66	5.31	0.985
	SE	1.43	0.001	0.07	1.54	0.004								
	Low 95% CI limit	89.4	0.039	1.69	57.8	0.116								
	Upper 95% CI limit	95.0	0.041	1.98	63.8	0.130								
Hoheria populnea	Value	70.5	0.025	2.67	50.2	0.069	120.7	38.2	21.0	49.2	10.3	-8.76	11.83	0.892
	SE	3.82	0.002	0.27	5.05	0.005								
	Low 95% CI limit	63.0	0.022	2.15	40.3	0.059								
	Upper 95% CI limit	78.0	0.029	3.2	60.1	0.080								
Pittosporum crassifolium	Value	91.2	0.046	1.41	46.7	0.167	137.9	81.9	11.7	46.7	5.7	-5.53	16.08	0.842
	SE	3.43	0.002	0.23	3.6	0.019								
	Low 95% CI limit	84.4	0.043	0.96	39.7	0.130								
	Upper 95% CI limit	97.9	0.049	1.86	53.8	0.204								
Salix schwerinii	Value	27.6	0.066	1.01	34.6	0.011	62.2	27.1	8.7	9.2	46.3	-22.42	13.21	0.417
	SE	3.62	0.013	2.2	26.37	0.005								
	Low 95% CI limit	20.5	0.042	-3.31	-17.1	0.000								
	Upper 95% CI limit	34.7	0.091	5.34	86.4	0.021								

RMSE, Root mean square error. MAPE, Mean Absolute Percentage Error. Adj R^2, Adjusted R squared.

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Table 7. Native (*Coprosma robusta, Griselinia littoralis, Hoheria populnea* and *Pittosporum crassifolium*) and exotic (*Salix schwerinii*) shrub species leaf *in vitro* fermentation kinetic parameters derived using dual pool model. Where, L, lag time (h); V_1, Fast pool total gas production (ml/g DM); V_2, Slow pool (ml/g DM); R_1, Fast pool rate of gas production (%/h); R_2, Slow rate (%/h), $V_{Sch,}$ total gas production, (ml/ g DM); $V_{1_{24}}$, total gas production for the fast pool after 24 hours (ml/g DM); $V_{2_{24}}$ slow pool after 24 hours (ml/g DM); $V_{1_{T_{0.5}}}$, fast pool total gas production half-life (h) and $V_{2_{T_{0.5}}}$, slow pool half-life (h)

Species	Parameters	V_1	R_1	L	V_2	R_2	V_{Sch}	$V_{1_{24}}$	$V_{1_{T_{0.5}}}$	$V_{2_{24}}$	$V_{2_{T_{0.5}}}$	MAPE	RMSE	Adj R^2
Coprosma robusta	Value	71.9	0.017	1.71	50.8	0.095	122.8	27.7	28.9	50.8	7.7	-4.87	6.83	0.938
	SE	3.16	0.001	0.17	0.67	0.003								
	Low 95% CI limit	65.8	0.016	1.38	49.5	0.089								
	Upper 95% CI limit	78.1	0.019	2.04	52.2	0.101								
Griselinia littoralis	Value	76.0	0.049	1.06	30.2	0.129	106.2	81.3	10.6	16.7	10.7	4.91	11.14	0.872
	SE	4.48	0.002	0.24	4.62	0.017								
	Low 95% CI limit	67.2	0.045	0.59	21.1	0.095								
	Upper 95% CI limit	84.8	0.053	1.53	39.2	0.162								
Hoheria populnea	Value	33.9	0.021	1.03	78.1	0.073	112.0	16.5	23.0	77.4	8.4	-6.15	10.93	0.876
	SE	2.16	0.003	0.21	2.77	0.003								
	Low 95% CI limit	29.7	0.016	0.62	72.7	0.066								
	Upper 95% CI limit	38.2	0.027	1.44	83.5	0.079								
Pittosporum crassifolium	Value	65.5	0.068	0.60	31.2	0.219	96.7	64.6	8.1	31.2	4.4	-2.11	6.70	0.922
	SE	2.06	0.002	0.13	2.11	0.019								
	Low 95% CI limit	61.4	0.065	0.35	27.1	0.181								
	Upper 95% CI limit	69.5	0.071	0.84	35.3	0.257								
Salix schwerinii	Value	56.2	0.014	1.42	32.6	0.097	88.8	32.5	7.8	18.0	36.5	-4.09	3.71	0.950
	SE	4.10	0.001	0.22	0.44	0.003								
	Low 95% CI limit	48.2	0.012	0.99	31.7	0.092								
	Upper 95% CI limit	64.2	0.015	1.86	33.4	0.103								

RMSE, Root mean square error. MAPE, Mean Absolute Percentage Error. Adj R^2, Adjusted R squared.

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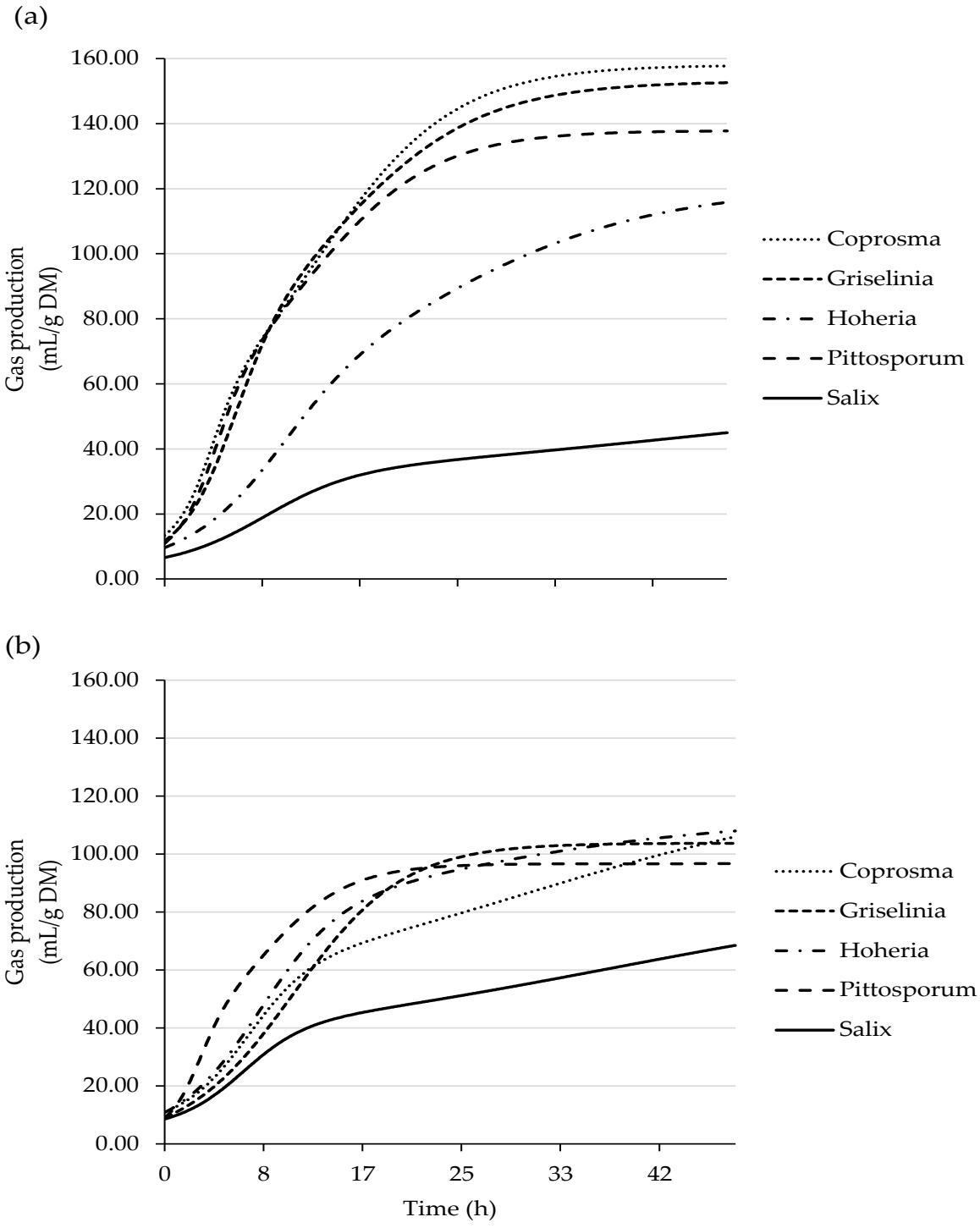

Figure 2. Predicted cumulative gas production curves over 48 hours for (a) leaves and (b) stems of native (*Coprosma robusta, Griselinia littoralis, Hoheria populnea* and *Pittosporum crassifolium*) and exotic (*Salix schwerinii*) shrub species using the dual pool model.

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3.3. *In vitro* fermentation end products

3.3.1. *Volatile fatty acids and microbial biomass*

The pH of the *in vitro* medium after fermentation ranged from 6.57 to 6.71 and differed (p<0.05) among species for leaves but not (p>0.05) stems (Table 8). Leaf pH was higher (p<0.05) in *S. schwerinii* than all other species, except *H. populnea*. For both leaves and stems, *S. schwerinii* had nearly twice the (p<0.05) MBM than the native shrub species, which did not differ (p>0.05).

The VFA varied (p<0.05) among species for leaves and stems, except for valerate in the leaves, and propionate and isovalerate in the stems. *Coprosma robusta* and *H. populnea* were similar (p>0.05) and had higher (p<0.05) *in vitro* medium acetate than *P. crassifolium* and *S. schwerinii*, which did not differ (p>0.05). *Salix schwerinii* leaves produced higher (p<0.05) propionate and lower (p<0.05) butyrate than the native shrub species However, *H. populnea* and *P. crassifolium* leaves were similar (p>0.05) and had higher (p<0.05) butyrate and valerate isomers than other species. Consequently, the ratio of acetate to propionate was similar (p>0.05) for *S. schwerinii* and *P. crassifolium* and lower (p<0.05) than in other species. The total VFA produced from *in vitro* fermentation of leaf material was similar (p>0.05) in the native shrub species and approximately four times higher (p<0.05) than in *S. schwerinii.*

Salix schwerinii stem *in vitro* medium had higher (p<0.05) acetate and lower (p<0.05) valerate than other species, except *C. robusta* which was not different (p>0.05) from any species. *Salix schwerinii* had lower (p<0.05) butyrate in the stem than the other species. However, isobutyrate was higher in *H. populnea* stem than other species, except for *P. crassifolium*. Total VFA production from *in vitro* fermentation of stem material was higher in the native shrub species (p<0.05) than in *S. schwerinii.*

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Table 8. pH, total volatile fatty acids (VFA) in millimoles (tVFA, mM), percentage of respective VFA (Acetate, Propionate, Isobutyrate, Butyrate, Isovalerate, Valerate, %), ratio of Acetate to Propionate (A:P) and microbial biomass in milligram per gram of dry matter (MBM, mg/ g DM) for leaves and stems for native (*Coprosma robusta, Griselinia litoralis, Hoheria populnea* and *Pittosporum crassifolium*) and an exotic (*Salix schwerinii*) shrub species with potential use as fodder sources in New Zealand

Shrub species	pH	Acetate	Propionate	Isobutyrate	Butyrate	Isovalerate	Valerate	Total VFA	A:P	MBM
Leaves										
Coprosma robusta	6.57^{b}	63.1^{a}	22.2^{c}	0.15^{b}	13.8^{a}	0.11^{b}	0.69	27.5^{a}	2.9^{a}	101.3^{b}
Griselinia litoralis	6.57^{b}	61.2^{ab}	23.8^{c}	0.00^{c}	15.1^{a}	0.00^{b}	0.63	27.6^{a}	2.6^{a}	80.2^{b}
Hoheria populnea	6.62^{ab}	62.7^{a}	25.0^{bc}	0.38^{a}	10.4^{b}	0.58^{a}	0.89	24.5^{a}	2.5^{a}	106.4^{b}
Pittosporum crassifolium	6.57^{b}	57.6^{b}	27.6^{b}	0.33^{a}	13.1^{a}	0.52^{a}	0.92	28.8^{a}	2.1^{b}	112.5^{b}
Salix schwerinii	6.70^{a}	58.1^{b}	33.9^{a}	0.00^{c}	7.9^{c}	0.11^{b}	0.75	7.3^{b}	1.7^{b}	260.2^{a}
Pooled SE	0.027	0.98	0.73	0.033	0.57	0.062	0.070	0.99	0.09	9.88
Stem										
Coprosma robusta	6.66	60.4^{ab}	25.0	0.00^{b}	14.2^{a}	0.00	0.74^{ab}	17.4^{a}	2.5	82.5^{b}
Griselinia litoralis	6.67	54.7^{b}	27.7	0.04^{b}	17.6^{a}	0.09	0.82^{a}	19.0^{a}	2.0	62.6^{b}
Hoheria populnea	6.66	55.8^{b}	27.9	0.29^{a}	15.1^{a}	0.38	1.03^{a}	19.9^{a}	2.0	70.1^{b}
Pittosporum crassifolium	6.67	53.3^{b}	31.9	0.09^{ab}	13.9^{a}	0.32	0.84^{a}	16.0^{a}	1.8	90.0^{b}
Salix schwerinii	6.71	68.6^{a}	25.9	0.00^{b}	7.7^{b}	0.00	0.29^{b}	9.6^{b}	2.7	144.4^{a}
Pooled SE	0.013	2.04	2.08	0.054	1.39	0.091	0.115	1.28	0.22	7.22

VFA, A:P and MBM with different superscripts in a column for the sample type are different at p< 0.05. Sample VFA value of 0.00 indicate the VFA was undetectable.

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3.3.2. *Fermentation greenhouse gases*

Fermentative production of the greenhouse gases, carbon dioxide (CO_2) and methane (CH_4), and carbon dioxide equivalent (CO_2 Eq) differed among the shrub species for both leaf and stem samples (Table 9). Production of greenhouse gases and CO_2 Eq was lower (p<0.05) in *S. schwerinii* than in the native shrub species for leaf material. Similarly, production of CO_2 from fermentation of stem material was also lower (p<0.05) for *S. schwerinii* compared to the native shrub species. Production of CH_4 from fermentation of stem material for *S. schwerinii* was comparable (p>0.05) to that of *C. robusta* and *P. crassifolium*, and in relation to production of CO_2 Eq, *S. schwerinii* stems were similar *P. crassifolium*.

Table 9. Native shrubs (*Coprosma robusta, Griselinia litoralis, Hoheria populnea* and *Pittosporum crassifolium*) and an exotic (*Salix schwerinii*) shrub species carbon dioxide (CO_2) and methane (CH_4) gas production in milliliters per gram of dry matter (mL /gDM) and green house carbon dioxide equivalent (CO_2 Eq) in grams per gram of dry matter (g/gDM) from the leaves and stems.

Shrub species	CO_2	CH_4	CO_2 Eq
Leaves			
Coprosma robusta	76.5^{ab}	46.0^{a}	0.77^{a}
Griselinia litoralis	76.3^{ab}	43.9^{a}	0.77^{a}
Hoheria populnea	64.2^{b}	37.8^{a}	0.66^{a}
Pittosporum crassifolium	77.7^{a}	41.3^{a}	0.74^{a}
Salix schwerinii	17.2^{c}	8.6^{b}	0.15^{b}
Pooled SE	3.01	2.07	0.039
Edible stem			
Coprosma robusta	47.6^{a}	26.7^{ab}	0.45^{a}
Griselinia litoralis	53.7^{a}	27.1^{a}	0.47^{a}
Hoheria populnea	54.7^{a}	28.0^{a}	0.49^{a}
Pittosporum crassifolium	42.1^{a}	20.9^{ab}	0.36^{ab}
Salix schwerinii	23.5^{b}	14.9^{b}	0.25^{b}
Pooled SE	3.54	2.76	0.031

Fermentation gas (CO_2 and CH_4) and carbon dioxide equivalent (CO_2 Eq) with different superscripts in a column for the sample type are different at p< 0.05.

4. Discussion

The objectives of the study were to determine the, (i) *in vitro* fermentation gas production, (ii) predict the *in vitro* fermentation kinetics using the single and dual pool models and (ii) to estimate the *in vitro* fermentation end products (volatile fatty acids and greenhouse gases) of four native shrubs with forage potential. Further, to compare the native shrubs to an exotic osier willow utilized in the North Island hill country sheep and beef farms. For purposes of discussion of the findings, the results sequence has been rearranged from the order used in the method and results sections.

4.1. Shrubs volatile fatty acids and microbial biomass production

Approximately 70% of the caloric requirements of ruminants are met by volatile fatty acids (VFA) produced by reticulorumen microbes [49]. However, the reticulorumen microbes metabolism and thus the quantity and proportions of VFA produced is affected by nutrients and non-nutritive factors in the diet [50,51]. *In vitro* fermentation of leaf and stem material from the native shrub species studied here, resulted in more than three times the amount of total VFA (tVFA) when compared to *S. schwerinii*. The high tVFA yield in the

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native shrub species suggests that their nutrients were more digestible and could supply more ME to the animal than *S. schwerinii.* Lower tVFA production during the *in vitro* fermentation of *S. schwerinii* may be due to the condensed tannins (CT), which are known to be present in Salix spp foliage [52–54]. However, the CT differences among species in this study were not measured and therefore this hypothesis cannot be tested and requires further attention.

Condensed tannins are complex polyphenolic compounds that bind to dietary proteins, polysaccharides, minerals and microbial endogenous proteins and enzymes, thereby retarding microbial growth and proliferation and hence production of VFA [55,56]. Although some pasture and fodder crops used in New Zealand contain small quantities of CT [57,58] which reduce *in vitro* VFA production [49], *S. schwerinii* foliage has been reported to contain higher levels (<50g CT/kg DM) [54,59]. Further, foliage CT concentration was found to be higher for *S. schwerinii* grown on the hill country than on the fertile flat and rolling lands in New Zealand [54], an environment that farmers are likely to plant native species. Comparatively, the tVFA produced by the native species (24.5 to 28.8 mM) was within range reported for perennial ryegrass-white clover pastures with up to 25% chicory (24.5 to 27.2 mM) [60], higher than for tropical shrubs (8.9 to 20.8 mM) [33] and lower than for leguminous shrubs (73.2 to 97.2 mM) [61], pasture grasses (perennial rye-grass, tall fescue, Yorkshire fog, phalaris and paspalum) leaves (112.1 mM) and stems (105.4 mM) [62] and ryegrass-white clover pastures with more than 25% chicory (29.8 to 33.4 mM) [60]

In vitro fermentation of native shrub species leaves produced higher tVFA than stems, which contrasted with that of *S. schwerinii*. This is likely due to the native shrub species having more highly fermentable carbohydrates in their leaves than their stems and is to be expected because stems contain higher levels of structural carbohydrates than leaves. The higher tVFA production from *in vitro* fermentation of *S. schwerinii* stems compared to leaves is likely due to the presence of CT in the leaves. [54] observed higher CT levels in the leaves of *S. schwerinii* compared to the stems.

The primary VFAs produced in the rumen are acetate, propionate and butyrate, with valerate and branched chain VFA's only found in small quantities [63]. The proportion of non-glucogenic (combined acetate and butyrate) VFA produced from *in vitro* fermentation of leaf and stem from the studied shrub species ranged from 66 to 76% of the tVFA. This is within typical ranges reported for forages (64 to 80%) in New Zealand [49,62,64,65], tropical shrubs (70.1 to 73.4%) [33] and leguminous (69.1 to 76.9%) and non-leguminous shrubs (73.4 to 79.6%) [61].

Proportionately, there was more acetate from the *in vitro* fermentation of the native species leaves than stems. In contrast, the *in vitro* fermentation of *S. schwerinii* stems produced greater amounts of acetate compared to the leaves. Acetate is a lipogenic VFA that results from fermentation of forage structural carbohydrates (ADF and NDF) and reduces with an increase in lignin content [55,63]. All shrubs had more fibre (NDF and ADF) in the stems than leaves. Further, lignin content was relatively lower in native species stems than leave but vice versa for *S. schwerinii.* Thus, typically more acetate would be expected after fermentation of the fibrous stems compared to leaves. However, the proportion of acetate after the *in vitro* fermentation of native species stems was lower than from the leaves. In addition, more butyrate and propionate resulted from *in vitro* fermentation of native species stems than leaves. This suggests that native species stems had higher readily digestible carbohydrates than the leaves. This is likely because the shrubs were in a vegetative state and only new growth stems (i.e., less than 5mm in diameter) were collected. Although not investigated, soluble and storage carbohydrates are typically high in new growth stems and their fermentation results in elevated butyrate and propionate VFAs, respectively [50,51,66,67].

In vitro fermentation microbial biomass (MBM) yield was higher in *S. schwerinii* than in the native species for both leaf and stem material and showed an inverse relationship to tVFA production. Production of VFA *in vitro* corresponds to growth and turnover of MBM and subsequent degradation of feed substrates [49]. However, the rate of growth

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and turnover of the microbes depend on nutrient supply from the host diet and is affected by non-nutritive and inhibitory factors that hinder organic matter digestibility [50,51]. In contrast to the native species, the high *in vitro* MBM and low tVFA yield observed in *S. schwerinii* suggests there was low growth and turnover of microbes. This may be due to the inhibitory effects of CT [54,62,68–70], which have been reported to be high in *S. schwerinii* [54,59]. Nutritionally, feedstuff with low digestibility have been shown to have low reticulorumen microbe turnover and hence reduced microbial protein supply to the animal [68,69,71].

4.2. *In* vitro gas production

The *in vitro* gas production was higher from native species than *S. schwerinii*. The lower *in vitro* gas production from *S. schwerinii* may be attributed to presence of CT. On average, the *in vitro* gas production from the native shrubs (112.5 to 131.2 mL/ gDM) was within the range previously reported for leguminous shrubs (113.7 to 148.5 mL/ gDM), higher than for non-leguminous shrubs (28.1 to 101.4 mL/ gDM) but lower than for *Moringa oleifera* (187.0 mL/ gDM) [61] and ryegrass (193.0 mL/ gDM) [66]. The shrubs' *in vitro* gas production was consistent with tVFA production and inverse to MBM yield. This supports earlier studies that have shown a positive corelation between *in vitro* gas production and tVFA [72,73] and an inverse relationship with MBM yield [71].

Native species' *in vitro* fermentation gas production was more from leaves than stems, in contrast to *S. schwerinii.* High *in vitro* gas production in the native species leaves can be associated to a higher production of acetate relative to butyrate and propionate VFAs [70], contrary to the stems. Stoichiometry of VFA proportions can be used to estimate the amount of gas production when feed substrates are fermented *in vitro* [71]. Fermentative formation of acetate has been reported to result in higher *in vitro* gas production [69,70], explaining the high *in vitro* gas produced from the native species leaves compared to the stems. Another factor that could have contributed to the higher *in vitro* gas production in the native species leaves, is the higher CP content of the leaves compared to the stems. Dietary CP provides reticulorumen microbes with nitrogen, which is essential for growth and proliferation, enhancing carbohydrate degradation, resulting in increased gas production [70].

Among the native species, *H. populnea* leaves had lower *in vitro* gas production despite having a higher CP. The lower *in vitro* gas production from *H. populnea* leaves can likely be explained by the higher ash content (11.6% DM) than in all other shrubs. Ash content suggests the presence of minerals which are inorganic and unfermentable [69]. Similar ash for *H. populnea* leaves has also been reported earlier [74,75] and was within range to that of forages [49], pasture grass (perennial rye-grass, tall fescue, Yorkshire fog, phalaris and paspalum) leaves (8.9 to 12.1% DM) but higher than for stems (5.5 to 8.9% DM) [62].

4.3. *In vitro* fermentation kinetics

Mathematical non-linear models are essential tools that can be used to describe *in vitro* fermentation gas production using parameters that have biological interpretation [14]. The models vary in complexity and differ in equation structure and parameters (pool or compartment) applied in predicting the *in vitro* fermentation gas production [13]. In this study, the single pool exponential model of [37] and dual pool logistic model developed by [11] were applied to fit the *in vitro* fermentation gas production. Both the models ranked the predicted *in vitro* fermentation gas production for the shrubs' leaves similarly to the measured *in vitro* fermentation total gas production. However, the models showed discrepancies in ranking of the predicted *in vitro* fermentation gas production for *C. robusta, G. littoralis* and *H. populnea* stems. The stems of these three species had similar *in vitro* fermentation total gas production measurement and therefore the discrepancies between the models are likely due to their fixed inflection points, which affected the predicted rate and asymptotic gas production [13]. However, both the single and dual pool

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models had good prediction accuracy (MAPE) and explained greater proportion of variability (Adjusted R squared) between the measured and predicted *in vitro* fermentation gas production for the shrubs except *S. schwerinii* leaves. The lower accuracy and higher variability for *S. schwerinii* leaves can likely be due to the inconsistently low gas production observed in the measured *in vitro* fermentation gas production.

Single pool model produced negative prediction for *in vitro* fermentation gas production from the immediately soluble fraction (*a*, mL/g DM) for the native species leaves. Similarly, negative *a* was predicted for the native species stems except for *C. robusta*. A negative *a* indicates negative gas production and is biologically incorrect. This showed the mathematical limitations of the single pool model in predicting the *in vitro* fermentation gas production for the native shrubs. Moreover, the single pool model numerically overestimated *in vitro* fermentation gas production of the native species except *H. populnea* stems. This can likely be attributed to the model assumption of a constant rate of fermentation [76–79], despite the native shrubs leaves having varying fermentable nutrients. Earlier studies have also demonstrated these limitations of using the single pool model in predicting *in vitro* fermentation gas production on feeds with mixed fermentable substrates [13,14,77].

The dual pool model predicted higher *in vitro* fermentation gas production and longer gas production half-life for the fast pool for the native species leaves in contrast to *S. schwerinii*. This observation suggests that the nutrients in the leaves of the native shrub species were readily fermentable, which is supported by the shrubs' observed nutrient composition, tVFA and total gas production in this study. However, the model predicted higher *in vitro* fermentation gas production from the slow pool for *H. populnea* stems. This was expected because *H. populnea* stems contained higher structural carbohydrates (NDF and ADF) compared to other shrubs. The higher *in vitro* fermentation gas production for fast pool and longer gas production half-life for the slow pool for *S. schwerinii* stems agreed with *in vitro* fermentation tVFA proportions and measured total gas production in this study. The coherence of the dual pool model in predicting the *in vitro* fermentation gas production for the studied shrubs supported earlier studies using different forages [3,9,13,14]. However, the model numerically underestimated *in vitro* gas production for all shrubs, except *C. robusta* leaves and *H. populnea* stems which it overestimated. Therefore, there is need for comparison of the dual pool model with other multicompartment models to determine the model that can best describe the *in vitro* fermentation gas production for the studied shrubs.

4.4. Greenhouse gases emission from the shrubs

Greenhouse gas production was higher for native shrub species than for *S. schwerinii*. In addition, native species leaves produced more greenhouse gases than stems, which was opposite for *S. schwerinii*. Proportionately, *in vitro* production of CH_4 gas was greater for native species' leaves than stems and vice versa for *S. schwerinii*. The CH_4 production from *S. schwerinii* was lower (14.9 mL /gDM) while native species (20.9 to 46.0 mL /gDM) were within ranges reported for New Zealand pastures (17.6 to 58.5 mL /gDM) [80]. The low enteric fermentation greenhouse gases production from *S. schwerinii* may be attributed to the presence of CT. Effects of CT in reducing enteric fermentation greenhouse gases production have been studied previously for forages [57,81] and leguminous and non-leguminous shrubs [61,82,83].

Carbon dioxide (CO_2) and methane (CH_4) are the major enteric fermentation greenhouse gases (GHGs) from ruminants [70]. Compared to CO_2 gas, CH_4 gas is the most important because it's more potent, equating to 25 carbon dioxide equivalents (CO_2 Eq) in global warming potential [70,73]. Further, enteric fermentation CH_4 gas accounts for approximately 84% of the gross CH_4 emissions in New Zealand [84,85]. However, the amount and proportions of enteric fermentation gases produced are dependent on the nutritional composition of ruminant feeds, as this influences reticulorumen microbial populations and fermentation pathways [70,86]. A higher acetate proportion results in

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higher CH_4 emissions [70,87,88], as was observed from the native species leaves. Acetogenesis causes release of hydrogen ions that are utilized by methanogenic reticulorumen microbes to reduce CO_2 thereby releasing CH_4 as metabolites [87,89]. Production of CH_4 gas by methanogenic microbes' results in 2 to 12% loss of energy from ingested feeds [87,88,90,91]. On the other hand, butyrate and propionate formation were elevated from *in vitro* fermentation of native species stems. Butyrate and propionate synthesis acts as hydrogen sinks and compete for the hydrogen ions with methanogenic rumen microbes thereby reducing CH_4 and promoting CO_2 production metabolic pathway [86,88] explaining the depressed CH_4 production from the *in vitro* fermentation of native species stems.

5. Conclusion

Findings from the current study show that fermentation of leaf and stem material from native shrub species resulted in higher *in vitro* total gas production than *S. schwerinii*. The single and dual pool models used to predict the *in vitro* fermentation total gas production for the shrubs had a satisfactory fit. However, the single pool model was biologically incorrect by predicting negative *in vitro* total gas production from immediately soluble fraction of native shrubs. On the other hand, the dual pool model better predicted *in vitro* fermentation total gas production and was coherent with measured *in vitro* fermentation end products. Native shrubs had higher production of volatile fatty acids from the *in vitro* fermentation of leaves and stems than *S. schwerinii.* Conversely, *S. schwerinii* yielded more microbial biomass from the *in vitro* fermentation of leaves and stems than the natives' species. The *in vitro* fermentation characteristics of the native species leaves, and stems suggest they were more digestible and could provide more energy and microbial proteins to animals compared to *S. schwerinii* if consumed. Comparing among the native shrubs, *H. populnea* leaves would be superior when consumed by providing higher crude protein and yielding lower *in vitro* fermentation total gas production and emitting lower greenhouse gases by volume. This study suggests that when consumed by ruminant livestock, native shrubs can provide adequate energy and microbial protein, and that greenhouse gas production from these species is generally within ranges reported for typical New Zealand pastures. Further studies are required to determine animal preference and intake and to quantify GHGs production *in vivo*.

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