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Pine afforestation improves the biological soil attributes linked to methane oxidation in a temperate zone of Argentina

Ezequiel Jesús Terán [1,✤], María Eugenia Priano [1,2], María Paula Juliarena [1,2], María Elena Fernández [3,4,5], Javier E. Gyenge [3,4,5]

[1] Centro de Investigaciones en Física e Ingeniería del Centro de la Provincia de Buenos Aires. Tandil, Argentina
[2] Facultad de Ciencias Exactas- Universidad Nacional del Centro de la Provincia de Buenos Aires, Buenos Aires, Argentina
[3] Consejo Nacional de Investigaciones Científicas y Técnicas, Argentina
[4] Instituto de Innovación para la Producción Agropecuaria y el Desarrollo Sostenible, Tandil
[5] International Associated Laboratory, Argentina- France

ECOLOGY

ABSTRACT

Background: Atmospheric methane (CH_4) is responsible for approximately 20% of global warming since the preindustrial era. Forests are land ecosystems whose role is crucial for mitigating the greenhouse effect due to their capacity to capture and store C and preserve other processes such as CH_4 oxidation in the soil. On the other hand, in the particular case of afforestation, there are contradictory results about the magnitude of CH_4 uptake variation due to changes in methanotrophic bacteria activity and its relationship with micro-environmental conditions.

Results: The average potential CH_4 oxidation rate in the laboratory (MOL) of afforested soil was 186% greater than that of the grassland, which could be marginally attributed to differences in soil physicochemical parameters like bulk density, pH and organic matter. A seasonal pattern in MOL was observed in both land uses, with the highest values at the warm and rainy season. MOL magnitude increased with soil depth up to 10-15 cm, which corresponds with the mineral layer.

Conclusion: Pine afforestation would improve the biological soil attributes linked to methane oxidising bacteria compared to the grassland systems.

Keywords: Land use change, Methanotrophic bacteria, methane uptake, GHG mitigation, ecological services

HIGHLIGHTS

Afforested soils showed the highest methane oxidation rates.
Methane oxidation rate was higher in the warm and wet season of the year.
The higher methane oxidation rates were obtained at 10-15 cm depth.
The methane oxidation rate variation was marginally explained by soil parameters

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✤Corresponding author e-mail: ezequiel.j.teran@ifas.exa.unicen.edu.ar
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INTRODUCTION

Atmospheric methane (CH_4) is responsible for approximately 20% of global warming since the preindustrial era (Kirschke et al., 2013). Because CH_4 oxidising bacteria (MOB) of the soils are responsible for the only known biological removal of atmospheric CH_4, the interest in MOB understanding is continuously increasing (Tveit, 2019). Communities of MOB are commonly present in a wide range of soils and sediments with different textures under dissimilar land uses and/or vegetation cover (Zeng et al., 2019; Judd et al., 2016; Serrano-Silva et al., 2014). Superimposed on soils characteristics, the seasonality of the precipitation and temperature impacts over MOB functionality, increasing their activity in relation with temperature and/or soil water content (Judd et al., 2016; Zeng et al., 2019). Moreover, within a particular site, land use may have a significant impact over MOB and their activity, but the mechanisms driving the effects of land-use changes are not well understood (Judd et al., 2016; Hiltbrunner et al., 2012).

Forests have been postulated as essential land ecosystems whose role is crucial for mitigating the greenhouse effect due to their capacity to capture and store carbon (C) and preserve other processes such as CH_4 oxidation in the soil (e.g. Gatica et al., 2020). In this regard, it is known that CH_4 uptake decreases when forests are converted into grassland or arable fields (Fest et al., 2017). This decrease has been attributed to the disturbance of soil physical characteristics such as compaction, which diminishes the CH_4 influx and oxygen (O_2) availability (Fest et al., 2015), affecting the ecological niche of methanotrophs (Hiltbrunner et al., 2012). However, the inverse land-use -afforestation- does not necessarily imply an increase in CH_4 oxidation, with observed positive and negative changes in net CH_4 uptake (Tate, 2015). This highlights the need for further field and laboratory measurements to disentangle the underlying mechanisms that explain the response variability.

Afforestation implies a drastic change in microenvironmental conditions along with changes in vegetation tissue chemistry entering the soil and, consequently, affecting soil life forms (Gonzales-Polo et al., 2019; Berthrong et al., 2012). However, the effects depend on the complex interaction between prior land use, climate and tree species planted (Gonzalez-Polo et al., 2019). One of the most common soil properties affected by afforestation with positive effects on soil methane oxidation is soil bulk density following by soil water content (De Bernardi et al., 2021), which usually decreases with stand age, improving aeration in soils and promoting the diffusion of O_2 and CH_4 (Bárcena et al., 2014). Tree species and canopy density affect soil water dynamics through rain interception and transpiration, which may, in turn, affect soluble salt and gas fluxes (Mujica et al., 2019; Bárcena et al., 2014). A decrease in soil water content is generally observed in forests compared to grasslands, which is associated with a higher potential CH_4 oxidation rate in the laboratory (MOL) and higher and different abundance of MOB (Xiangyin and Groffman, 2018; Tate, 2015; Bárcena et al., 2014; Hiltbrunner et al., 2012). Also, afforestation changes the amount and quality of soil organic matter, and other soil properties such as pH, with proportional changes in microbial biomass population and/or activity (De Bernardi et al., 2021; Tate, 2015).

Radiata or Monterey pine (*Pinus radiata* D. Don) is one of the most cultivated pines in the world, occupying almost 4.2 mill. ha in several countries (Mead, 2013). In Argentina, *P. radiata* is one of the main tree species planted in hilly areas of the Pampean region, but plantations with this species could also be found in other regions, such as Patagonia. Previous studies have shown that *in situ* soil CH_4 uptake fluxes under *P. radiata* plantations were several times higher than those measured in contiguous grassland and annual crops (De Bernardi et al., 2021). These differences in CH_4 uptake showed a strong correlation with soil water content and bulk density, suggesting that soil diffusivity was one of the main drivers of this process. However, the contribution of MOB on these processes needs to be assessed.

Our objective was to describe the relationships between the CH_4 oxidation capacity (MOL) under laboratory conditions and soil variables (soil water content, bulk density, organic matter, pH) in samples taken in a grassland and contiguous afforested land in different dates and soil depth. We hypothesized that the spatio-temporal variation in MOL is explained mainly by the variations in soil variables induced by land-use change. We also expected a common seasonal variation in both land uses driven by macroclimatic conditions.

MATERIAL AND METHODS

Site description and soil sampling

The experimental field study is located in the surrounding rural zone of Tandil city, Argentina (37° 33.598' S, 59° 7.824' W, 180 m.a.s.l). The climate of the region is temperate with a homogeneous distribution of the precipitation during the year and < 22°C of average temperature during the warm period of the year (cfb following Kóppen-Geiger classification; Ferrere et al., 2015). The annual average temperature is 13.7 °C, the annual average precipitation is 889 mm/yr (Servicio Meteorológico Nacional de Argentina; SMN).

The field site consisted of two adjacent areas, a *P. radiata* plantation (afforestation) and the natural grassland, placed on a hill slope (Figure 1). The soil is a black *Hapludoll*, with sandy loam texture in the upper layer (0-10 cm; 15 /18% clay, 30 /18 % silt and 56 / 64% sand for soils in the grassland /afforestation, respectively), increasing the content of clay at the deeper soil layers (Milione et al., 2020; Ferrere et al., 2015). The presence of rock outcrops characterizes the landscape, and the solum thickness ranges between 10 cm at the top of the hill and 50 cm at the piedmont. The area with *P. radiata* (Af) was planted in 2001 (17 years old at the start of the present study). Despite different tree cover level is observed across the whole afforested area (stand of around 22 ha.), the sampled plot has homogeneous conditions with average tree density of 727 plant.ha^{-1} pruned to 4 m height. This tree cover allows for the development of a grass understory with 60–90% cover, dominated by *Dactylis glomerata* at the moment of the measurements (De Bernardi et al., 2021). The natural grassland (Gs), a sector representing the original vegetation system at the piedmont, covers 0.9 ha and comprises several species, with *D. glomerata* being the dominant one. Still, there are other gramineous and rosaceous species such as *Bromus catharticus, Paspalum spp., Leontodon taraxacoides* and *Cirsium vulgare*. This grassland has native species, such as *Paspalum quadrifarium*, as well as exotic but naturalized ones, such as *D. glomerata*.

Soil samples at four layers (00–05, 05–10, 10–15, 15–20 cm depth) were bi-monthly collected during one year (June 2018 to June 2019) from at least three random points in each land use. The sampled soil at each date, soil depth and land-use, was mixed to obtain a composite sample representing soil heterogeneity from each soil layer. Soil samples were stored at 4 °C in dark conditions after fieldwork until being analyzed in the laboratory (Price et al., 2003). Air temperature was measured during the sampling time.

Soil variables

Soil analyses were performed immediately after the field samplings from subsamples of the obtained composite samples. Gravimetric soil water content (SWC; %) at field conditions was determined by weighting fresh and dry soil samples (see below). To this end, samples were oven dried for 24 hours at 105 °C. Soil water holding capacity (WHC; %) was obtained by flooding 100 g of dry soil with deionized water and leaving it to drain by gravity until drainage ceased. Then, samples were weighed and SWC and WHC were calculated according to equation [1]. Where D_r= humid or drained soil mass and D = dry soil mass (Burt, 2004). After that, SWC was reported as relative to WHC_0.

$$SWC \text{ or } WHC_0 = (D_r - D)\, D^{-1} \qquad \text{(Eq. 1)}$$

Organic matter (OM; %) was determined by Loss of Ignition (LOI) method proposed by Schulte and Hopkins (Eyherabide et al., 2014) drying 5 g of sieved soil samples (500 μm mesh) for 24 hours at 105 °C. After dry weight (*D*) was recorded samples were calcinated until constant weight at 360 °C (*C*). OM was calculated as is shown in (Eq. 2).

$$OM = (D - C)\, D^{-1} \qquad \text{(Eq. 2)}$$

Soil pH was determined by suspension of soil in decarbonated-distilled water (1:2.5, w:v; Deng et al., 2011; Burt, 2004) and measured by a pH-meter (Trans instruments HP3040; Singapore). Bulk density (BD; g cm^{-3}) was calculated by Adams equation (Adams, 1973): $BD = 100[\%OM\ 0.244^{-1}+(100-\%OM)\ MBD^{-1}]^{-1}$, where, MBD represents the mineral bulk density whose typical value is 1.64.

Figure 1. Field site and studied plots. Aerial photograph of the study zone, showing both *Pinus radiata* afforestation (Af) and Grassland (Gs), relative location (top, source: Google Earth Pro 2021). Photographs of each plot Gs (middle) and Af (bottom).

Potential CH_4 oxidation rate under laboratory conditions

Fresh soil from each layer was homogenized by sieving (2 mm) and moistening to achieve a water content of 50 % of water holding capacity (WHC; Serrano-Silva et al., 2014). All layers were subsampled (200 g) into triplicate and distributed in 2,01 L static hermetic chambers with a one-way stopcock for the headspace's syringe sampling. A blank was performed for each layer, consisting of autoclaving a soil subsample (0.5 h at 122 °C). The samples were stabilized in the dark at 21 °C and in contact with atmospheric air overnight; then, the chambers were opened under ambient air conditions for 30 min and closed before the start of the measurements (Bárcena et al., 2014; Hiltbrunner et al., 2012).

Potential MOL was estimated by monitoring the CH_4 concentration decay in the chamber's headspace along with five-time steps: 0, 1, 2, 3 and 4 hours after the chamber closure. An exponential regression was fitted to the data. The calculations were considered valid when the determination coefficient (R^2) was ≥ 0.75 (Price et al., 2004).

Gas concentrations were analyzed with a gas chromatograph (Agilent 7890A; United States of America), equipped with a 1.8-m Poropak Q (80/100 mesh) column and a FID to determine CH_4 concentration. The carrier gas (N_2) pressure was maintained at 27 psi, and flame gases (H_2 and O_2) were set at 40 and 450 ml min^{-1}. The oven and FID detector temperatures were 60 °C and 300 °C, respectively.

Statistical analyses

Nonlinear regression analysis was applied to determine the relationship between MOL in each site to two climatic variables shared by both land-uses that vary with the sampling date (precipitation and the average daily air temperature). The precipitation value corresponds to the sum of all precipitation events between the current and the previous sampling date. The adjusted models for each relationship (MOL vs the climatic variable) were compared between the two land-uses using F tests (n = 7 for each environmental variable, Neter and Wasserman, 1974). ANOVA and LSD Fisher were employed to determine statistical differences in the data set of each soil layer at each land use.

A principal component analysis (PCA) was carried out to study samples' clustering concerning the studied variables that potentially vary with the land-uses (OM, pH, and SWC). Then, to identify quantitative relationships between MOL and those soil variables, multiple linear regressions analyses (MLR) were carried out, analysing both land uses together (n = 45) and separately (Gs, n = 24; Af, n = 21). Generalized Linear Models (GLM) were employed to determine if any of the measured soil variables were significant in the explanation of MOL variations detected (n = 45) when categorical variables are included (Land use, Soil layer, and Sampling month). R software (R Core Team, 2019) was employed for all the analyses, and a significance level of at least $p < 0.05$ was considered. The figures were drawn using OriginPro, 2006.

RESULTS

Mean MOL values and their relationship with the climatic conditions

As expected in the study region, located in the Southern hemisphere, the highest mean monthly temperature was reached in January (20.8 °C) and the lower one in July (7.2 °C). On the other hand, the rainiest month was March (93 mm of accumulated precipitation), and the driest one was July (43 mm of accumulated rain). The study period was relatively warmer than the mean historical data, with a temperature deviation up to +0.5 °C. The precipitation deviation was lower than 5 % of the historical monthly average data over the whole studied period (from reports of SMN, 2018).

In all field sampling dates, the Af plot showed higher potential MOL values than Gs, reaching maximum values of about four times than those from Gs (Figure 2 a and b), mainly during the warmest season. Different asymptotic relationships (Figure 2 a and b) were found comparing land-uses between potential MOL vs. precipitation and vs. air temperature, which differed between Af and Gs (F test, p<0.05).

The first soil layer (00-05 cm) presented the lowest potential MOL values (Figure 3). Nevertheless, Af average potential MOL at this first soil layer was five times higher than that at Gs, reaching values of 1.0 ± 0.5 and 0.2 ± 1.3 nmol CH_4 g^{-1} h^{-1}, respectively. Deeper layers from Gs were similar between them but lower than Af deeper layers (Figure 3). Maximum values were recorded at 10-15 cm soil layer in both land-uses. The deepest layer (15-20 cm) registered a sharp decrease of potential MOL compared to the rest of the soil layers.

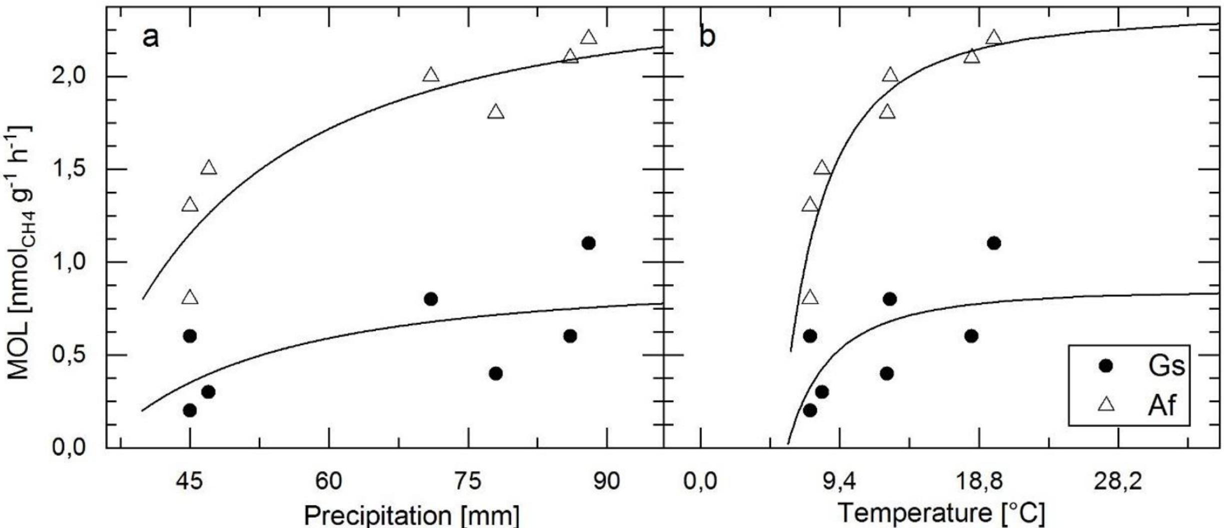

Figure 2. Regression models between averaged MOL and precipitation (a; Af: Eq. $y=(-2611.248/(x-2.4426))^{1/2}$, R^2=0.83, p<0.01; Gs: Eq. $y=(-1106.4651/(x-0.8982))^{1/2}$, R^2 = 0.39, p < 0.01) and air temperature (b; Af: Eq. $y=(-67.4140/(x-2.3391))^{1/2}$, R^2=0.99, p<0.01; Gs: Eq. $y=(-29.1030/(x-0.8596))^{1/2}$, R^2=0.39, p<0.01) at both afforestation (Af) and grassland (Gs) use.

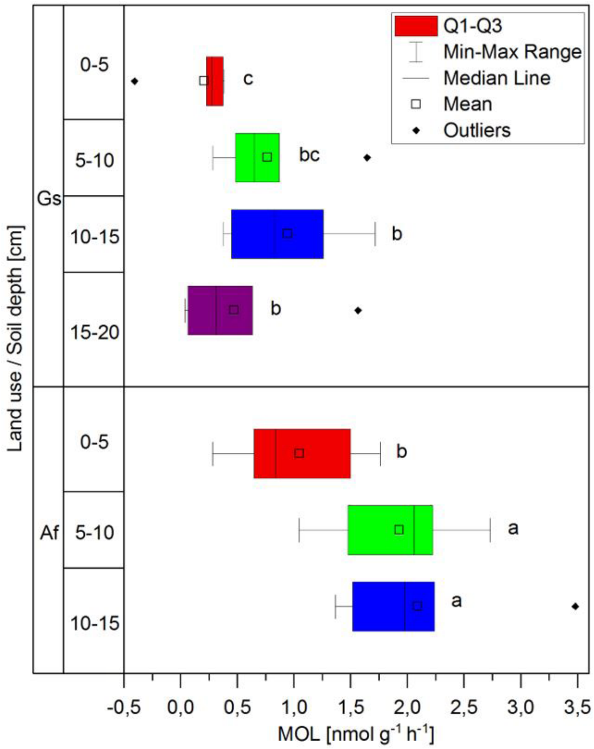

Figure 3. Box plot of MOL at each soil layer and land use. Af: Afforestation; Gs: Grassland. Common letters indicate no statistical differences (n=7).

Relationships between MOL and soil variables

In general, pH increased while OM and SWC decreased with soil depth (Table 1). The variation in measured variables (MOL, OM, and pH) across land uses and dates was primarily captured by the first two principal components (PC 1 and PC 2) in the PCA performed for all soil layers, i.e. 71.54% of the variation (Figure 4). The lower components were not essential and had eigenvalues lower than 0.6. In this analysis, PC 1 explained 44.87 % of the total variance and clustered the sampling points along this axis for each land-use, according to their soil physicochemical characteristics (Figure 4). It is important to note that Af and Gs points share similar responses to the soil variables; that is, their clusters are barely separated in the PCA. Thus, the PC1-axis may be interpreted as the 'soil physicochemical variation between land-uses'. The pH (PC1 eigenvector = -0.60) was the parameter with the highest loading on PC 1, followed by the OM (PC1 eigenvector = -0.54). The interpretation of the PC2 axis was more straightforward, i.e. the highest loadings came from SWC (PC2 eigenvector = 0.62) and MOL (PC2 eigenvector = -0.50) and it captures in part the variation in SWC and CH_4 oxidation along soil depth (Figure 4). CH_4 oxidation is highest in medium and deeper layers of Af (blue and green triangles in Figure 4) where SWC is minimum. In contrast, it can be observed that the upper soil layer from both land uses clusters together in an opposite position to the deeper soil layers, where SWC is maximum and MOL is minimum. So, the PC2-axis might be interpreted as 'soil depth' in SWC and MOL variation.

The soil physicochemical variables showed statistical significance with potential MOL when MLR and GLM were applied to find dependencies between them (Table 2). When pooled data of both land uses in stepwise MLR were considered, SWC was the only parameter that had a significant influence on MOL (p = 0.038). This relation between potential MOL and SWC is quite expected, given the large difference in mean SWC between land uses (ΔSWC = 8.0 %DW). On the other hand, in the Gs model (data of this land-use alone), the parameter with the highest significance level was the pH (p = 0.015), probably because of the variation of this parameter along the soil profile (ΔpH = 1.1). The model did not find significant effects of soil variables on the Af land use. However this model presented the highest determination coefficient but with no significance at 0.05 level (R^2 = 0.35; p = 0.061). Finally, the best GLM model, with a determination coefficient of 0.86, included qualitative factors such as sampling date, land-use, and soil depth. It shows not only the significance of some quantitative parameters (OM, p = 2.02 E^{-5}; pH, p = 0.08), but also the high influence of qualitative factors (land-use, p = 1.30 E^{-9}; sampling date, p = 1.61 E^{-8}; soil depth, p = 1.68 E^{-6}) on MOL explanation (Table 2).

Table 1. Mean and standard deviation of soil variables (n = 7 given by the different sampling dates), in the different soil layers of the grassland (Gs) and afforestation (Af) land. pH: acidity, OM: organic matter, SWC: soil water content and BD: bulk density.

Land use	Soil depth (cm)	Soil variables			
		pH	OM (%)	SWC (%)	BD (g cm^{-3})
Gs	00-05	6.6 ± 0.4	15 ± 2	49 ± 9	0.88 ± 0.07
	05-10	7.3 ± 0.6	12 ± 3	47 ± 10	1.0 ± 0.1
	10-15	7.6 ± 0.6	10 ± 4	41 ± 8	1.1 ± 0.1
	15-20	7.7 ± 0.5	9 ± 3	39 ± 10	1.1 ± 0.1
Af	00-05	5.6 ± 0.2	18 ± 1	36 ± 8	0.81 ± 0.03
	05-10	5.9 ± 0.2	13 ± 1	35 ± 6	0.93 ± 0.03
	10-15	6.3 ± 0.5	12 ± 6	36 ± 6	0.97 ± 0.02

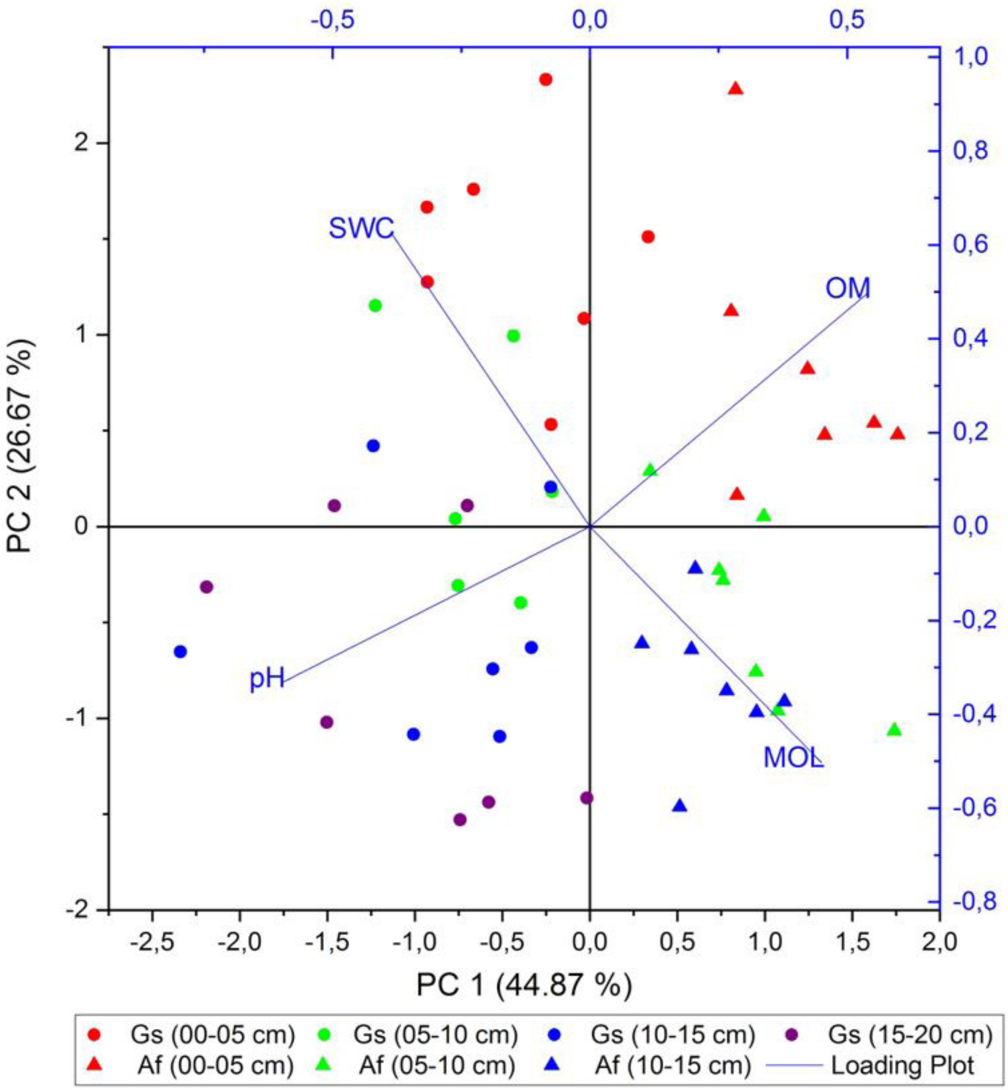

Figure 4. Bi-plot of the principal component analysis (PCA) for MOL and soil variables measured in two land uses: Af: Afforestation and Gs: Grassland. Vectors represent variables, and triangles and circles represent samples. OM: organic matter content, SWC: soil water content relative to water holding capacity of the sample. Different colour dots and triangles represent the different soil layers.

Tab.2 Multiple linear regression (MLR) and generalised linear model (GLM) equations relating MOL and soil variables considering both land-uses together and separately. Af: Afforestation; Gs: Grassland. OM: organic matter content, SL: soil layer. Month abbreviations indicate when the field sampling was carried out.

Model type	Land use	Model expression	R^2 (p-value)
MLR	Both	MOL = 3.106* + (0.00742 OM) - (0.151 pH) - (0.0263 SWC*)	0.15 (0.068)
	Both	MOL = -2.646• + (0.0392 OM) + (0.422 pH*) - (0,00351 SWC)	0.29 (0.043)
	Af	MOL = 0.435 - (0.108 OM) + (0.382 pH) + (0.0158 SWC)	0.35 (0.061)
GLM	00-05	MOL =[5.0184 - (0.122 OM***) - (0.149 pH•) + (0.004 SWC) - (0.773 Af***) - [1.013 SL(05-10)***] - [1.132 SL(10-15)***] - [1.278 SL(15-20)***] - (0.331 Aug*) -	0.86 (N.A.)
		(0.447 Oct**) - (0.346 Dec*) - (0.502 Feb***) - (0.472 Apr**) - (0.058 Jun)]-1	

N.A.: not applicable. Significance codes: '***' : 0.001; '**' : 0.01; '*' : 0.05; '•' : 0.1.

DISCUSSION

The present study analysed the differences in CH_4 oxidation rates in soil samples coming from a natural grassland and a contiguous afforested land, where changes in CH_4 fluxes were previously reported (De Bernardi et al, 2021). These differences might be due to changes in physical processes affecting CH_4 movement into the soil -such as gas diffusivity-, to changes in the biological (microbial) activity of soils, or both. To complement the field measurements reported in De Bernardi et al. (2021), the present study analysed on the biological aspects of CH_4 fluxes by means of measuring the potential CH_4 oxidation rate (MOL) under laboratory-controlled conditions, of soil samples taken in those land uses along a year. We hypothesized that MOL would be different between land uses, increasing towards the afforested land, due to changes in soil parameters that affect microbial development, and that besides those differences, MOL would vary along the year in response to seasonal climatic conditions. Our results support this hypothesis, and highlight that, in addition to the physical processes affecting the observed CH_4 fluxes, the biological MOB activity could play a role explaining the increased CH_4 uptake observed under pine plantations compared to grassland. In this sense, the literature explains that vegetation cover plays a key role in CH_4 oxidation potential of the soil, however, the direction of the change observed is not always the same (Shukla et al., 2014). Our results are in line with Shukla et al. (2014) who proposed that a reforested or afforested soils generally showed higher CH_4 oxidation potential than a grassland one. Hütsch (1998) found that MOL was 11 times higher in a forest soil which has been undisturbed for at least a century than that measured in a field under different cultivation methods (from no-till to the more traditional methods). The estimated MOL in our sites, from 0.2 to 2.2 nmol CH_4 g^{-1} h^{-1} agreed with those estimated in several environments. Gulledge et al. (2004) found that the MOL in a pine afforestation located in Massachusetts USA, was close to 1 nmol CH_4 g^{-1} h^{-1} in the upper 5 cm of soil depth. Zeng et al. (2019) reported daily MOL values close to 0.005 nmol CH_4 g^{-1} h^{-1} in a temperate coniferous (*Pinus armandii, Quercus aliena*) forest. Price et al. (2003) found the highest MOL (1.67 nmol g^{-1} h^{-1}) at the 05-10 cm of soil depth in a 3000-5000 years-old coniferous forest of New Zealand. Hiltbrunner et al. (2012) found maximum TOM values in a subalpine region between 0.067 and 0.069 nmol CH_4 g^{-1} h^{-1} from a pasture and a forest soil (Norway spruce), respectively.

In both studied land uses, the higher values of MOL occurred during the warmer and wetter season of the year (Figure 1), as it was reported in several temperate ecosystems (Sihi et al., 2020). Soil MOB abundance and activity may increase during warm periods due to the positive temperature effect on both the enzymatic processes and the transport of CH_4 in the gaseous phase (Praeg et al., 2017). In general, in order to characterize the potential MOB activity for a particular land use, MOL measurements are carried out only once a year (Fest et al., 2015). However, based on the observed seasonal behaviour, when a MOL range is required, it is important to determine it, at least, during the most contrasting climatic seasons, which are commonly the warmest and coldest months (Zeng et al., 2019; Bárcena et al., 2014; Prajapati and Jacinthe, 2014; Serrano-Silva et al., 2014).

As it was postulated by several authors, CH_4 uptake rates are different between ecosystems mainly due to variations in physical soil properties (bulk density, soil water content) that affect the gas diffusion process and/or the actual or potential bacteria activity related to CH_4 oxidation (De Bernardi et al., 2021; Wu et al., 2020; Zeng et al., 2019; Serrano-Silva et al., 2014). Accordingly, we found different MOL values between land-uses (higher in the afforested land than in the grassland), that are correlated with soil variables that favour gas diffusion, such as SWC (Figure 4). At the same time, MOL varied with the soil depth in the upper 20 cm indicating that MOB populations change even in response to small changes in soil attributes, as was also documented in other ecosystems (Bárcena et al., 2014; Prajapati and Jacinthe, 2014; Price et al., 2003). Several studies have analysed the MOL variation along soil depth. Generally, the reported profiles show the highest MOL in the upper 10 cm of soil depth, close to the atmosphere-soil interphase (between 00 and 10 cm depth), and then the rates decrease until MOL value is nearly zero below 40 or 50 cm depth which is probably link with the reduction of methane availability that occurs in depth (Shukla el al., 2014; Price et al., 2004). On the other hand, Adamsen and King (1993) found a MOL profile more similar to our results, that is, with a pick of MOB activity at intermediate depths of 5-15 cm.

Our results show that the measured soil variables can explain marginally the observed MOL variation comparing both land uses and soil layers (Table 2). The models of Table 2 indicated that the effect of SWC was significant only when data of both land-uses were pooled together, increasing the range of SWC values (Table 1). Mean SWC was generally lower in the afforested land than in the contiguous grassland, which may be explained by both the higher rain interception and high evapotranspiration that characterize forests compared to herbaceous vegetation (Mujica et al., 2019; Bárcena et al., 2014; Hiltbrunner et al., 2012). It is important to note that trees have also influence over the water fluxes beyond the deepest soil layer analyzed in this study (Mujica et al., 2019; Bárcena et al., 2014), and this can also alter the distribution and activity of the methanogenic bacteria beneath these layers. Our laboratory study could isolate the activity of methanotrophic bacteria, which are aerobic, but the CH_4 fluxes in the field are the result of the activity of both the methanotrophic and the anaerobic methanogenic bacteria. In this regard, Priano et al. (2017) observed an increment in methane concentration at 30 cm of soil depth (in a pasture and a mixed deciduous grove) associated with an increase in clay content in this soil layer, which could favour anoxic conditions and also indicate the presence of methanogens. However, considering the upper layers where methanotrophs are more abundant, studies in afforested areas with *Eucalyptus* spp. and *Pinus* spp. in the Pampa region showed that the upper 20 cm of soil under the trees remain drier than in the grasslands during most of the year, except during a few days that correspond with heavy rainy days (De Bernardi et al. 2021; Mujica et al., 2019). This contribution of trees to soil drying facilitates gases (O_2 and CH_4) diffusion and as a consequence, modify MOB metabolism (Tiwari et al., 2015), abundance and/or taxonomic group (Judd et al., 2016; Kravchenko and Sukhacheva, 2017; Zeng et al., 2019). In relation to this, Trentini et al. (2020) showed that soil water content influenced microbial community in forest soils and Feng et al. (2021) demonstrated that the relative abundance and diversity of methanotrophs decreased when moisture increase in soils incubations at 15 and 30°C.

On the other hand, within land uses, the most important soil variables associated with MOL were pH and OM. As it occurs with pH, OM influence on potential MOL was largely linked to the spatial variation of this factor, but when it was analyzed across the soil layers (i.e. the different soil depths) instead of between land-uses (Figure 4). Changes in OM depend on the characteristics of the vegetation cover in interaction with management which can affect MOL by limiting the total mineralisable carbon that is available for sustaining microbial activity (Tate, 2015). In our case, the OM content at the first soil layer (00-05 cm) was the highest across the soil profile (Table 1), but MOL was the lowest registered (Figure 3). This observation demonstrates that there are other variables or processes that limit MOL at the first soil layer since several authors have pointed out the positive effect of OM over MOL. This positive effect is related to the facilitation to methanotrophs provided by OM retention of moisture and ensuring its supply during periods of water deficit; by promoting the gas transport by diffusion through the soil; and by indirectly increasing CH_4 concentration in the soil by providing metabolic substrates for methanogens activity (Tate, 2015; Shukla et al, 2014). On the other hand, pH had a higher effect in Gs than in Af, probably due the relatively high vertical variability (at different soil layers) of pH in this land-use, which was not observed in the afforested land (Table 1 and 2). Our results indicate that the decrease of pH in the afforested soils, that seems like a general pattern in the world (Berthong et al., 2009), do not negatively compromise the functioning of MOB. More effort is needed in order to determine the effect of single and multiple soil factors over MOB diversity and activity to manage these productive systems from a sustainable point of view. Finally, another potential driver of MOL activity, not considered in our study, is the soil temperature (both absolute and its variations in depth). Soil under *Eucalyptus* spp. plantations in the studied region presented higher temperature values during winter and similar values during the summer than the contiguous grassland soil surface (Mujica et al., 2020). In this regard, there might be other edaphic factors -not measured here- and/or additive or synergistic effects between two or more environmental variables that modify the activity and/or diversity of MOB at micro- (soil layers) and meso- (land use) environmental levels (Price et al., 2004). Soils conditions under tree cover showed several differences that, in turn, facilitate the functionality of the MOB under field and laboratory conditions (MOL) as we proved in our study. However, more effort is needed in order to understand the variables that promote MOB activity and the increased provision of ecological services contributing to the role of forest plantations for greenhouse gases mitigation.

CONCLUSIONS

Our results indicate that the time of the year and the depth of the soil could be important in order to compare MOL values of soils under different land uses. Moreover, pine afforestation can improve the biological soil attributes linked to CH_4 uptake in temperate regions compared to original grassland. Future studies should add variables not studied here, integrating vegetation, geochemical and microbiological information over a longer time period, to build a comprehensive framework for understanding and modelling soil CH_4 uptake in different land uses.

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Database: EJT, MEP.
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