Facultat de Ciències

Memòria del Treball de Fi de Grau

Methane emissions and production from tree stems of *Quercus suber* in a Mediterranean forest

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Nom estudiant: Roger Fíguls Calvelo

Correu electrònic: roger.estaron@gmail.com

Grau en: Biologia

Nom del tutor: Josep Barba Ferrer

Correu electrònic: josep.barba@udg.edu

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Agraïments

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RESUM

Els arbres de boscos alts (amb sòls ben drenats) poden tenir la capacitat d'emetre CH_4 a través dels troncs. Les emissions derivades dels arbres poden provenir d'una font del terra o ser produïdes en els seus teixits per arqueus metanògens que hi habiten. Tanmateix, hi ha poca informació sobre com els possibles orígens varien segons l'espècie. No s'han realitzat estudis sobre emissions de CH_4 d'arbres en ecosistemes limitats per aigua, com els mediterranis. En aquest projecte s'estudien les emissions de CH_4 derivades del tronc de l'alzina surera (*Quercus suber*), una espècie àmpliament distribuïda a la conca mediterrània. L'escorça d'aquesta espècie (suro) s'extreu per a fins comercials degut a la seva impermeabilitat. En aquest treball, s'avalua l'efecte de l'extracció del suro (pelat) en les possibles emissions del tronc, ja que el suro pot actuar com a barrera física per a la difusió del CH_4 cap a l'atmosfera.

Es mesuren les emissions de CH_4 del tronc d'arbres pelats i no pelats a dues alçades, una a la zona d'extracció del suro (part inferior del tronc) i una altra per sobre (zona no pelada). Es van realitzar mesures durant cinc campanyes properes a la temporada de pela. Aquestes emissions es correlacionen amb el diàmetre a l'alçada del pit (DBH) i el contingut volumètric d'aigua del sòl (VWC). També es van prendre mostres de fusta per avaluar la seva capacitat de produir CH_4 en incubacions anaeròbiques de laboratori.

Els resultats demostren que l'alzina surera és capaç d'emetre CH_4 i, a més, tenen alts fluxos en comparació amb altres estudis (59.83 $\mu mol\ m^{-2}\ h^{-1}$ de mitjana), correlacionats positivament amb el DBH. Els fluxos mostren un patró vertical molt marcat a través del tronc, sent més alts a la base de l'arbre. Això podria ser degut a la composició i propietats del suro a la part inferior, ja que tots els arbres de l'estudi van ser pelats fa 12-14 anys. La capa extreta es regenera a un suro més prim i fracturat. Suggerim que la capa regenerada pot ser més permeable que l'original, sent menys resistent a la difusió de gasos. Sorprenentment, no es van trobar diferències entre arbres pelats i no pelats, ni tan sols tres mesos després d'extreure el suro, la qual cosa suggereix que la capa de suro regenerada pot tenir gairebé la mateixa resistència al flux de CH_4 que l'absència de la capa. El VWC tampoc influeix en el flux, fet que pot indicar que probablement la producció del sòl no sigui la font del CH_4. També es va trobar producció de CH_4 en els tres teixits per a tots els arbres (sense diferència entre arbres pelats i no pelats), fet que suggereix que el CH_4 emès va ser produït per aquests.

RESUMEN

Los árboles de bosques altos (con suelos bien drenados) pueden tener la capacidad de emitir CH_4 a través de los troncos. Las emisiones derivadas de los árboles pueden provenir de una fuente del suelo o ser producidas en sus tejidos por arqueos metanógenos que habitan en ellos. Sin embargo, hay poca información sobre cómo los posibles orígenes varían según la especie. No se han realizado estudios sobre emisiones de CH_4 de árboles en ecosistemas limitados por agua, como los mediterráneos. En este proyecto se estudian las emisiones de CH_4 derivadas del tronco del alcornoque (*Quercus suber*), una especie ampliamente distribuida en la cuenca mediterránea. La corteza de esta especie (corcho) se extrae con fines comerciales debido a su impermeabilidad. En este trabajo, se evalúa el efecto de la extracción del corcho (saca) en las posibles emisiones del tronco, ya que el corcho puede actuar como barrera física para la difusión del CH_4 hacia la atmósfera.

Se miden las emisiones de CH_4 del tronco de árboles pelados y no pelados a dos alturas, una en la zona de extracción del corcho (parte inferior del tronco) y otra por encima (zona no pelada). Se realizaron mediciones durante cinco campañas cercanas a la temporada de saca. Estas emisiones se correlacionan con el diámetro a la altura del pecho (DBH) y el contenido volumétrico de agua del suelo (VWC). También se tomaron muestras de madera para evaluar su capacidad de producir CH_4 en incubaciones anaeróbicas de laboratorio.

Los resultados demuestran que el alcornoque es capaz de emitir CH_4 y, además, tienen altos flujos en comparación con otros estudios (59.83 $\mu mol\ m^{-2}\ h^{-1}$ de media), correlacionados positivamente con el DBH. Los flujos muestran un patrón vertical muy marcado a través del tronco, siendo más altos en la base del árbol. Esto podría deberse a la composición y propiedades del corcho en la parte inferior, ya que todos los árboles del estudio fueron pelados hace 12-14 años. La capa extraída se regenera a un corcho más delgado y fracturado. Sugerimos que la capa regenerada puede ser más permeable que la original, siendo menos resistente a la difusión de gases. Sorprendentemente, no se encontraron diferencias entre árboles pelados y no pelados, ni siquiera tres meses después de extraer el corcho, lo que sugiere que la capa de corcho regenerada puede tener casi la misma resistencia al flujo de CH_4 que la ausencia de la capa. El VWC tampoco influye en el flujo, lo que puede indicar que probablemente la producción del suelo no sea la fuente del CH_4. También se encontró producción de CH_4 en los tres tejidos en todos los árboles (sin diferencia entre árboles pelados y no pelados), lo que sugiere que el CH_4 emitido fue producido por estos.

ABSTRACT

Upland trees can have the capacity to emit CH_4 through the stems. Tree-derived emissions can result from soil source or be produced in tree tissues by methanogenic archaea inhabiting the trees. However, there is still limited information on how the different origins depend on different species or environmental conditions. No studies of tree CH_4 emissions have been done in water-limited ecosystems such as Mediterranean ones. Here we present a study on stem derived CH_4 emissions from cork oak (*Quercus suber*), a species well distributed across the Mediterranean basis. The bask of this species (cork) is commonly extracted for business, since it has insulation characteristics. We assessed the effect of cork removal (peeling) on the potential stem emissions, since cork may be acting as a physical barrier for methane diffusion from the stem to the atmosphere.

We measured CH_4 stem emissions of peeled and unpeeled trees at two different heights, one on the cork extraction zone (bottom part of the stem) and the other above it (unpeeled zone). Measures were made on five campaigns around peeling season. We correlated these emissions with diameter at breast height and soil volumetric water content (VWC). We also took tree cores to assess their capacity to produce CH_4 under laboratory anaerobic incubations.

Our results prove that cork oaks were not only capable of emitting CH_4 but also had high fluxes compared to other studies (59.83 µmol m^{-2} h^{-1} on average), positively correlated with DBH. Fluxes had a very strong vertical pattern through the stem, being higher on the base of the tree. This could be due to the composition and physical properties of the cork at the lower part of the tree, since all trees in our study were peeled 12-14 years ago. The extracted layer regenerates to a thinner and more fractured cork. We suggest that the regenerated layer may be more permeable than the original one, being less resistant to gas diffusivity. Surprisingly, no differences were found between peeled and unpeeled trees, not even three months after extracting the cork, suggesting that the regenerated cork layer may have almost the same resistance to CH_4 flux as the absence of the layer. VWC did not influence the flux either, suggesting that soil production was unlikely to be the methane source. Moreover, CH_4 production was found in all three tissues for all trees (with no difference between peeled and unpeeled trees), suggesting that the emitted CH_4 was produced by tree tissues.

REFLECTIONS

Ethics

This project focuses on the effect of the cork layer extraction from cork oaks (*Quercus suber*). The study side is managed by the Consorci de les Gavarres. The Gavarres massif is important for the cork industry, which involves many businesses (Kim et al., 2017). Because of this, our study can have conflict of interest regarding the importance of the cork industry. To avoid it, our project approach is the most objective as possible, focusing only on the greenhouse gases exchanges between the tree and the atmosphere. Moreover, our results might have an important impact on this investigation field as well as in climate change knowledge. Is because of these that we have big responsibility during the project.

Environmental sustainability

The study site of this project is in property managed by the Consorci de les Gavarres in Cassà de la Selva, Girona. This property is 30 minutes away by car from the College of Sciences of University of Girona. During the project, there was not a good management of the rides since multiple cars were used in each campaign, increasing pollution. Nevertheless, this project studies methane emissions from cork oak, closely related to climate change. Therefore, it can provide new perspectives to this research field since plant-based methane emissions contribute ca. 5-22% to the global methane emissions (Carmichael et al., 2014).

Gender

This TFG is a project of the research group “Sòls i Vegetació en la Mediterrània” of the environmental science department on the University of Girona (UdG) and is entirely composed of women. Dra. Maria Dolors Verdaguer, Dra. Maria Assumpta Gispert and Dra. Laura Llorens are the head researchers of the group. The UdG faculty of science incudes different degrees in which woman have great student representation (more than 50% in all degrees) (Universitat de Girona). Even though 46% of researchers in this faculty are woman, few women have top ranks jobs (Universitat de Girona), causing the glass ceiling effect. It is duty for all scientist to fight against this bias and make a difference of it.

INTRODUCTION

Plant-based methane emissions contribute ca. 32-143 Tg CH_4 year^{-1} (5-22%) to the global CH_4 budget (Carmichael et al., 2014). Upland trees (i.e. growing in free-drained soils) could be an important source of those emissions, contributing ca. 0.4% (Wang et al., 2021) to the total ecosystem fluxes. Moreover, methane has a warming potential 32-45 fold than CO_2 (Neubauer & Megonigal, 2015), so emissions from trees might have a huge effect on the global climate and atmospheric chemistry.

Although upland forests CH_4 emissions are lower than the ones in wetland forests (Covey & Megonigal, 2019), methane can accumulate in upland tree trunks at high concentrations (Covey et al., 2012). Methane emissions in living or dead trees ranges between 17000 µmol m^{-2} h^{-1} and 0.7 µmol m^{-2} h^{-1} (Covey & Megonigal, 2019). These emissions can differ within different species and may be positively correlated with diameter at breast height (DBH) (Pitz et al., 2018). Other variables such as soil temperature or soil volumetric water content (VWC) can affect methane emissions too (Pitz et al., 2018).

Methane trunk emissions could potentially be produced in soils under anoxic conditions (Pitz et al., 2018) or within the heartwood of trees (Wang et al., 2016; Yip et al., 2019). On one hand, there are studies that suggest a soil derived CH_4 production, in upland forests (Maier et al., 2018). On the other hand, high water content or high wood density in trees induces anoxic conditions for CH_4 microbial production in the heartwood (Wang et al., 2016). This methane production could result from acetate fermentation or CO_2 reduction (Conrad, 2005), being the second one the dominant pathway (Whiticar, 1999). The methanobacteriaceae archaea family (methanogenic archaea) seems to be the main responsible of this CH_4 production, being *Methanobacterium* the most dominant OTUs within woody tissues (Yip et al., 2019; Zeikus & Henning, 1975). Anaerobic bacteria can be found too in the heartwood layer of trees (Yip et al., 2019).

Methane can be transported through the tree passively or actively. In upland forests, trees do not have aerenchyma, hence passive transport is driven by diffusion within heartwood tissue and direct horizontal diffusion for vertical and radial transport respectively (Barba et al., 2019). Active transport on upland trees is guided by sap flow (Barba et al., 2019). Moreover, other tree structures such as lenticels facilitate the radial transport of the gas (Covey & Megonigal, 2019), even though there is a certain resistance of this radial diffusion (Wang et al., 2016). On wetland forests, passive transport is driven by aerenchyma whereas active transport by pressurized ventilation or convective throughflow (Barba et al., 2019).

To our knowledge, there are no published studies on plant mediated CH_4 emissions in Mediterranean upland forests (Covey & Megonigal, 2019). However, Barba et al. (*unpublished results*) found, in a Mediterranean ecosystem (Alt Empordà, Spain), high stem CH_4 emissions from cork oak (*Quercus suber*) (despite the data came from just one day of field measurements and from a limited number of trees). However, the mechanisms, drivers nor the sources of those emissions as well as their presence in other Mediterranean forests are still unclear. Studies of CH_4 emissions have found positive relationships between soil water content or proximity of water table to the surface, being important drivers of the methane fluxes (Machacova et al., 2016; Pitz et al., 2018; Terazawa et al., 2015; Wang et al., 2016; Yip et al., 2019). However, our study site was characterized by drought conditions [Figure 1], hence, we would expect low or negligible methane fluxes.

Cork oak is a typical Mediterranean species located between 300-600 m above sea level (Gil & Varela, 2008). It has a longevity of 200-250 years and its canopy height average is 15-20 m (Gil & Varela, 2008). Moreover, this species produces cork, an important raw material for many businesses (Kim et al., 2017), due to the impermeable feature to gas and liquids (Gil, 2009). Even though trees are frequently peeled, *Quercus suber* can regenerate the cork layer as long as the vascular cambium is not damaged (Oliveira & Costa, 2012).

When the tree is 30 years old, it is peeled for the first time (Kim et al., 2017). It takes 9 to 12 years for the cork layer to regenerate until 30 mm width, when it can be peeled again (Bugalho et al., 2011). The peeling season is between May and June, when the layer is released causing the minimum damage to the tree. The new cork layer starts growing 25-35 days after the peeling (Pereira, 2011), and since the Mediterranean climate is characterized by drought summers (Lionello et al., 2006), the effect of the extraction can be affected during this period.

The study site was located in a property managed for cork extraction business. Trees in our study had not been peeled for the last 12-14 years, but cork layers had been extracted at least once in each tree (Q. Gubau, personal communication). Cork extraction is done at the bottom part of the tree, from a height three times the diameter at breast height (DBH). Moreover, the extraction is only done in trees with more than 20 cm of DBH.

Measuring stem emissions at different heights might be important, since a decrease in methane emissions with stem height could be a result of a soil source of the gas (Pitz et al., 2018). Assuming a soil source and a positive correlation between soil moisture and CH_4 production, our fluxes should be low due to the high drought period during the study [Figure 1]. However, variability with stem height could also result from the cork extraction. Comparison between peeled and unpeeled trees shall determine the effect of cork extraction. Chamber measurements were made in different campaigns (before, during and after the peeling of the cork) to assess the effect of cork extraction in a temporal scale. DBH can be an important factor to take into consideration as well, hence there could be a positive correlation between methane emissions and overall DBH (Pitz et al., 2018) and heartwood diameter (Wang et al., 2017).

OBJECTIVES

Because of the lack of information on tree stems CH_4 from semi-arid places, and the very limited understanding of the cork oak CH_4 emissions (Covey & Megonigal, 2019), the principal aim of this study is to determine whether this species emits CH_4 and which effect on CH_4 emissions might have the peeling process. Due to the drought conditions of our study, we hypothesize that the fluxes will be low or negligible, since soil moisture is usually an important driver of the emissions (Machacova et al., 2016; Pitz et al., 2018; Terazawa et al., 2015; Wang et al., 2016; Yip et al., 2019).

The second aim of the study is to evaluate the effect of the cork extraction on the methane emissions, since cork might block its transport through the atmosphere. We expect that the cork-extraction may result in an increase of stem CH_4 emissions to the atmosphere as a result of decrease of radial diffusivity resistance.

Due to the early regeneration of the cork layer (25-35 days after peeling) (Pereira, 2011), we intend to assess the immediate effect of the extraction during the peeling process, one week, one month and three months after. Because drought conditions during the summer (Lionello et al., 2006) might affect the fluxes, campaigns overlap with this season.

We also intend to establish the correlation between CH_4 emissions and other variables. Since soil moisture is usually positively correlated with stem emissions (Machacova et al., 2016; Pitz et al., 2018; Terazawa et al., 2015; Wang et al., 2016; Yip et al., 2019), we expect to find low stem CH_4 emissions in this water-limited ecosystem. We expect, however, positive correlation between DBH and methane fluxes (Pitz et al., 2018).

Moreover, we intend to bring some light on the source of the stem derived methane (soil or heartwood production). Conditions in our study are characterized by drought period and soil source is usually linked to moisture (Machacova et al., 2016; Maier et al., 2018). Hence, we expect to find heartwood production instead of a soil source.

MATERIALS AND METHODS

SITE DESCRIPTION

The experiment was carried out at Can Vilallonga (41.88N, 2.91E; Cassà de la Selva, Girona), a mixed Mediterranean forest owned by the Institut Català del Sòl and managed by the Consorci de les Gavarres for cork harvesting. The climate is Mediterranean, with a mean annual temperature of 15.14ºC and an annual precipitation of 401.7 mm (Servei Meteorològic de Catalunya). Therefore, our experiment was characterized by harsh drought conditions [Figure 1], from two years before the experiment. The experimental area was ca. 100 m^2, and was divided in two blocks by a dry stream (less than 50 m away from each other), with similar slope and soil conditions.

Cork oak (*Quercus suber*) was the most abundant arboreal species, and in less abundance was evergreen oak (*Quercus ilex*). The understorey community was dominated by strawberry tree (*Arbutus unedo*), Montpellier cistus (*Cistus monspeliensis*) and tree heather (*Erica arborea*).

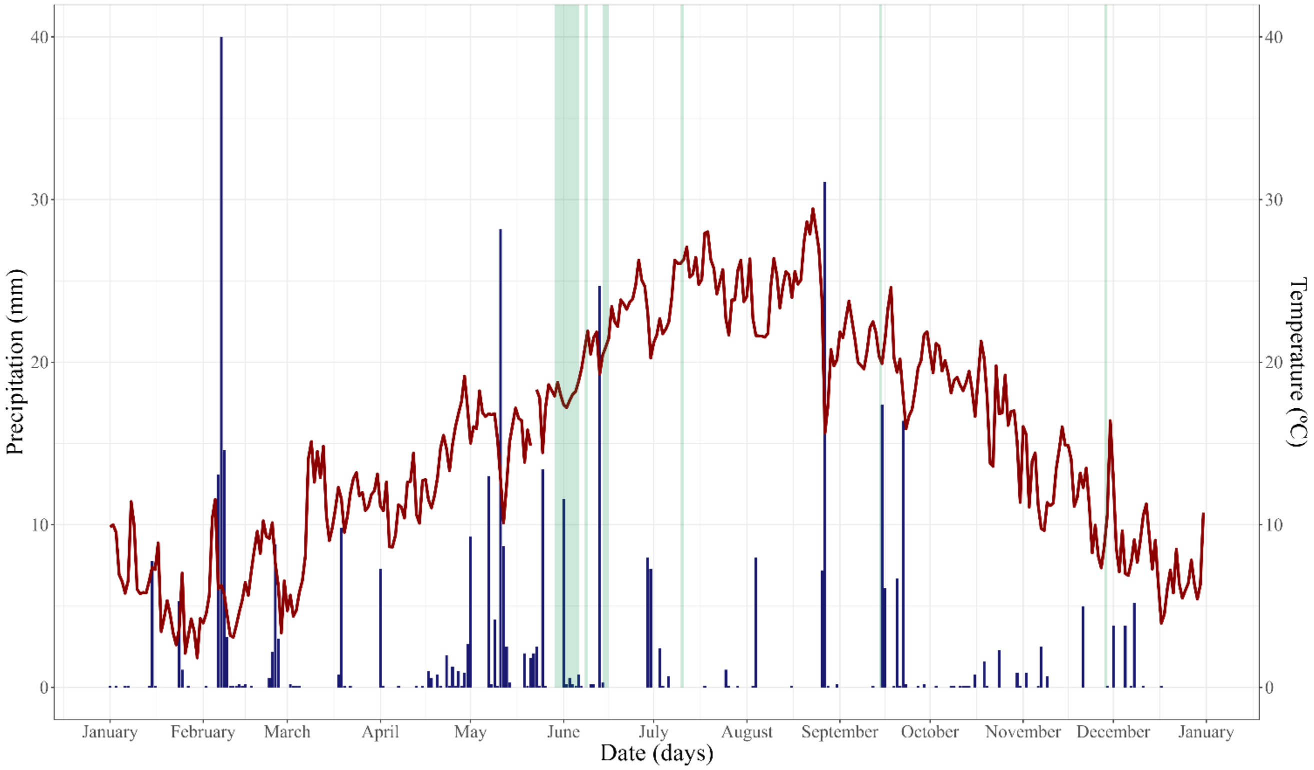


Figure 1. Daily precipitation and average daily temperature during the year of the study from a nearby meteorological station (41.88N, 2.91E) (Servei Meteorològic de Catalunya). Campaigns are highlighted in brown.

EXPERIMENTAL DESIGN

In this study, we measured stem CH_4 emissions from 40 cork oaks (11 of those on the right plot and 29 on the left plot). Twenty-two of those trees were peeled on June 8th and 9th (from now on, Treatment), whereas the other 20 were kept unmanaged for the entire experiment (Control). Both groups of trees were equally balanced in diameter at breast height (DBH) (between 17.5 cm and 41.1 cm, 26.2 in average). Moreover, none of the trees have had the cork removed in 12-14 years. The Treatment oaks were peeled from the ground to three-times-diameter height [Figure 2].

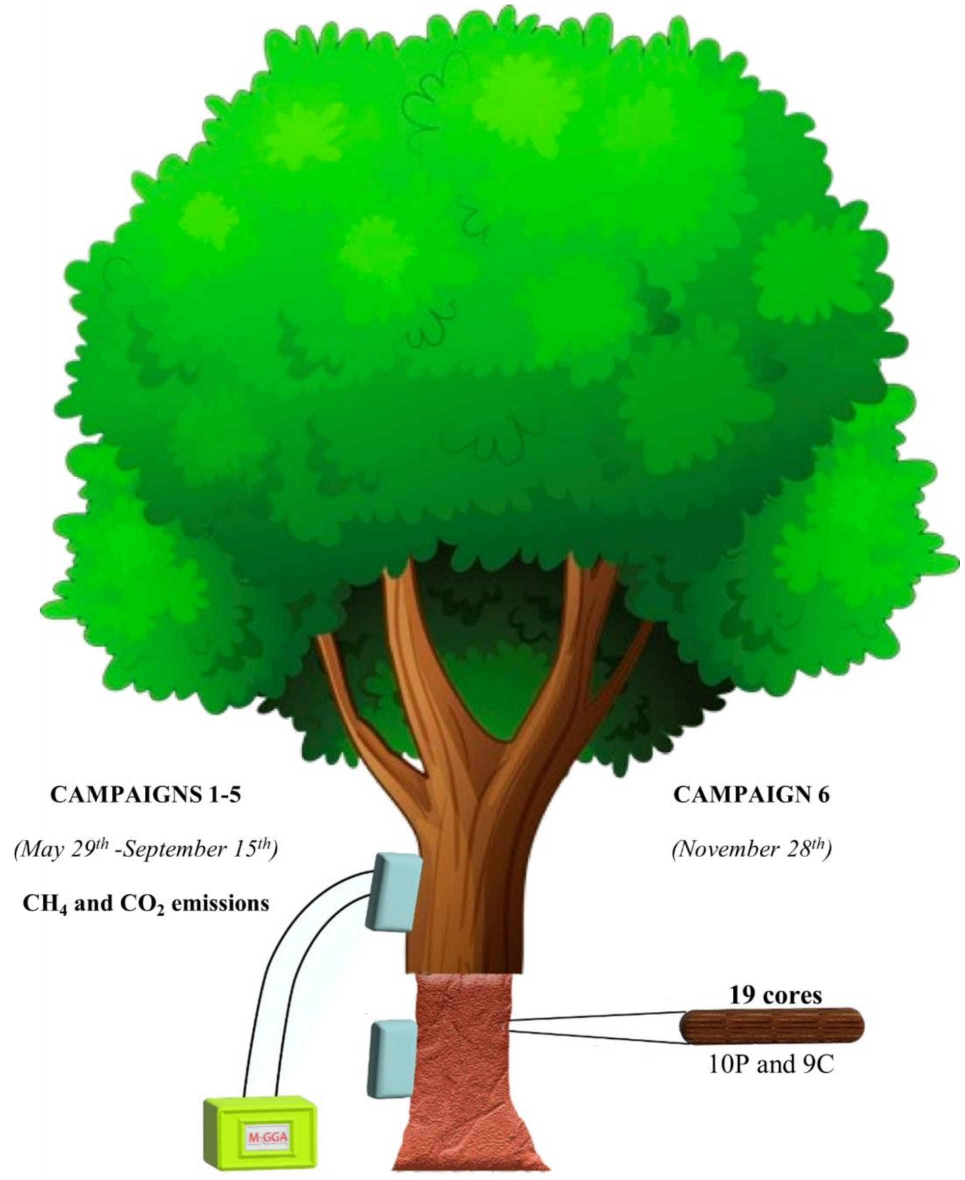


Figure 2. Campaign organization during the project. On Campaigns 1 to 5, CH_4 emissions were measured using a portable greenhouse gas analyser (M-GGA, Los Gatos Research, California). During these campaigns, soil temperature and volumetric water content (VWC) were measured too for each tree. On campaign 6, tree cores were extracted from 19 trees to assess the wood-methane production capacity, through incubations. In this example, the tree represents a peeled tree. P stands for Peeled trees and C for Control trees.

METHANE FLUXES MEASUREMENTS

Methane emissions from trunks were measured in 5 different campaigns [Figure 2]. The first campaign was performed on May 29th, June 5th and 6th, one week before the cork extraction, so all the trees measured for that campaign were unpeeled. Flux measurements in the second campaign were measured in the first 10 minutes after performing the cork extraction, on June 8th and 9th. The third and fourth campaigns were performed, one week (June 14th, 15th and 16th) and one month after the cork extraction (July 10th and 11th). The last campaign was three months after the cork extraction (September 14th and 15th) [Figure 6].

CH_4 emissions were measured at two tree heights, to study the effect of height and cork extraction. Bottom part measurements were made at ~ 50 cm height from the ground whereas upper part measurements were made at ~ 150 cm height. Cork extraction was done only on the bottom part of treatment trees [Figure 3], so bottom measurements for the peeled trees were performed without the cork, whereas upper measurements for peeled trees were performed on top of the cork. Therefore, comparing bottom measurements from control and treated trees enabled comparison of the effect of the cork extraction on the CH_4 fluxes, whereas comparing bottom and upper measurements of the same trees was used to determine CH_4 flux vertical pattern.

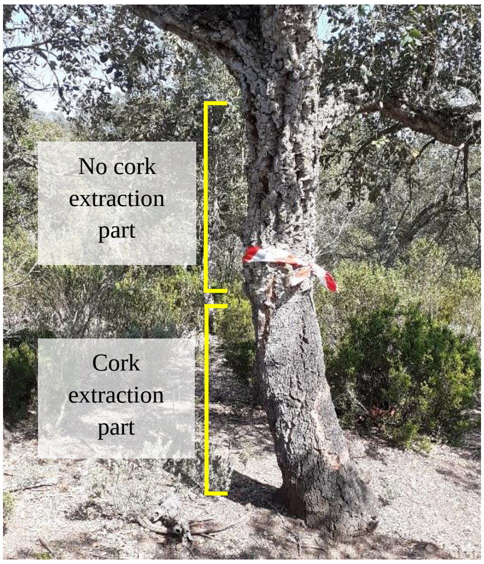
Soil temperature and soil Volumetric Water Content (VWC) were measured in all the campaigns around a 2 m radium from the tree stem, at 10 cm depth, simultaneously the gas emission measurement. For the soil temperature and VWC, we used a digital soil thermometer (HH806WE, Omega, Taiwan) and a digital moisture meter (TDR 300 Soil Moisture Meter, FieldScout, Pennsylvania, EUA), respectively. For each tree, two measures of soil temperature and three of VWC were registered.

Gas analyser

CH_4 emissions were measured using a portable gas analyser (M-GGA-918, ABB Inc, Quebec, Canada). M-GGA quantifies concentrations of CO_2 (1 – 20000 ppm), CH_4 (0.01 – 100 ppm) and water vapor (500 – 70000 ppm) from the measured trees, by absorption spectrophotometry. Plastic chambers connected to the M-GGA by PVC tubes (polyvinyl chloride) were used to measure the gas emissions from trunks. The plastic chambers were hermetically sealed to the tree with clay, which does not interfere with the CH_4 and CO_2 measurements (Jeffrey et al., 2020) [Figure 4]. Chambers of two different sizes (area of 341 cm^2 and 540 cm^2) were used depending on the tree diameter.

The gas analyser registers one concentration of gas per second from the inside the chambers and returns it into the chamber, creating a closed system in which emissions from the tree accumulates inside the chamber. Each stem flux measurement lasted 5 minutes.

Flux measurement quality was assessed visually in real time, checking the steady increment of gas concentrations inside the chamber, which denotes the proper sealing of the camber to the tree [Figure 5].



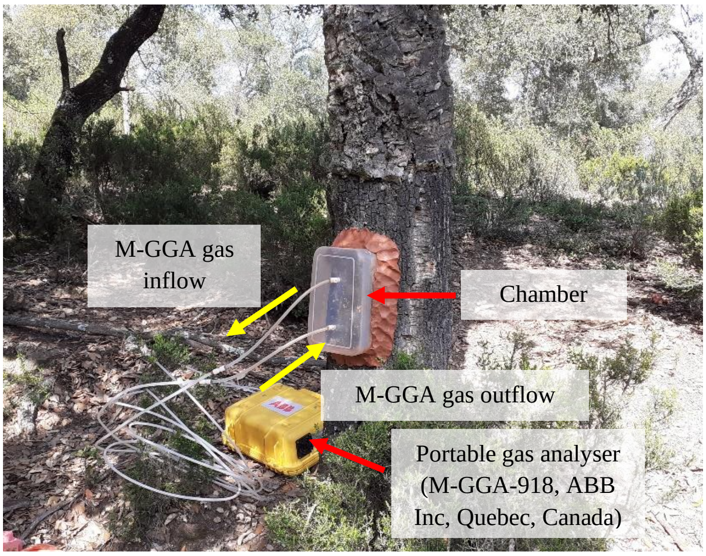


Figure 3. CH_4 measures zones of a control tree (unpeeled tree). The cork extraction part is ~ 50cm height whereas the no cork extraction part is ~ 150cm height. Both control and treatment trees have the same parts but in control trees there is no cork extraction.

Figure 4. CH_4 measures in no cork extraction part of a control tree. The portable gas analyser (M-GGA) and the chamber are pointed in red arrows. The gas flux through the M-GGA and the chamber is pointed in yellow arrows.

Fluxes data processing

CH_4 concentration increment within the chamber during each measurement was used to estimate the stem fluxes, using the following equation (Pumpanen et al., 2004):

$$F = (dC/dt) * (Vc/Ac) * P/((R*(T+273.15))),$$

were *F* being the gas flux, *dC/dt* is concentration over time (ppm^{-1}), *Vc* is the system volume, *Ac* is the measured area (chamber size), *P* is atmospheric pressure, *R* is the gas constant value ($0.008314\ kg\ m^2\ \mu mol^{-1}\ K^{-1}\ s^{-2}$), *T* is the temperature (ºC) and *273.15* is Celsius to Kelvin conversion factor.

Chamber stable conditions (well-sealed chambers) were needed to process emissions measurements. Assuming a constant gas flux during each measure, the concentration increment should be constant, and because of this, it has a linear slope. For CO_2 measures, emissions are always remarkably high, with a lineal slope of the concentrations during the measured period. If the R^2 of the linear fit was above 0.9 for CO_2 measurements, chamber internal conditions were considered stable, whereas lower R^2 values were indicators of unstable chamber conditions. Accordingly, fluxes with CO_2 R^2 lower than 0.9 were discarded. For CH_4 measurements, there were few cases where fluxes were so close to 0 that the R^2 was close to 0 as well. However, we did not discard such fluxes if the R^2 for CO_2 was higher than 0.9 (stable conditions).

Methane fluxes were calculated using the *goFlux* package of R (Rheault et al., 2024). Fluxes were calculated using two different approaches: linear regression (LM) and Hutchinson and Mosier method (HM) (Hutchinson & Mosier, 1981). Some fluxes do not fit with LM models due to not lineality. HM regressions, on the contrary, could better fit with non-linear concentrations, avoiding flux overestimation if the case. The package *goFlux* calculates the flux using both approaches, keeps the best flux based on the AICc statistics and gives an index of model fit of each approach based on AICc statistics.

Once the CH_4 fluxes were calculated, a linear mixed model was performed using the *lmer* function from the *lme4* package (Bates et al., 2015). Measurement height, treatment (peeled or control), number campaign, soil temperature, VWC and DBH were added as fixed factors. Because the effect of treatment may change as we move forward of the cork removal campaign, interactions between campaign and treatment, and campaign and height were added to the model. Additionally, measurements from the first campaign, when neither treated nor control trees were peeled, were included as fixed factors. Finally, tree identity was included in the random part of the model to account for the temporal autocorrelation of the measurements. In order to achieve model residuals normality, CH_4 fluxes were transformed using the Yeo Johnson transformation (Bishara & Hittner, 2012). Post-hoc comparisons were performed using the *emmeans* function from *emmeans* package (Lenth, 2024).

To better understand the model, interaction between treatment and height was not added to the final model, since this interaction was not significant. Moreover, the effect of the plot variable was not interesting for our study, but it had to be considered as a random effect. To simplify the model, plot was treated as a fixed effect to detect if it affected the fluxes. Because it was not significant, the plot variable was not added to the model neither as a random nor fixed variable.

Furthermore, negative fluxes were discarded to match model normality assumptions, which represented less than 3% of the data. Finally, we also excluded an extreme high flux that was an order of magnitude higher than the second highest flux (excluded flux: 2439.32 nmol m^{-2} s^{-1}).

TREE CORE INCUBATIONS

Methane production from cork oaks was studied using core incubations (Covey et al., 2012; Pangala et al., 2017). On November 28th, one tree core was extracted from the bark to the pith on the bottom part (~ 50cm height) of 19 trees (10 treatment and 9 control), at a perpendicular angle using an increment borer (5mm). Each core was split into sapwood (SW) and heartwood (HW) and cork (CORK) (the latest only available for Control oaks) and enclosed in different incubation jars provided with a rubber septa (47 samples).

Once a sample was sealed, and within the first minute after core collection, the inner space of the incubation jars was fumigated with nitrogen gas to keep the samples in anoxic conditions. Later in the day, and once in the laboratory, the same fumigation process was done again to ensure the initial CH_4 concentration was zero.

Incubation measurements were made 24h and 48h after the core sampling, using the M-GGA coupled with a closed gas system loop of 91.2 mL using PVC tubes and a syringe with a septa [Figure 5]. Ten mL of gas was sampled from each incubation jar and injected into the loop using a syringe, while increasing the volume of the system 10 mL to keep a constant pressure on the system.

The difference between the concentration in the loop before and after the gas injection was used to calculate the core methane production. Whenever gas samples were extracted from the incubation jar, 10 mL of N_2 were added to keep its pressure constant. CO_2 concentration and production was calculated too in order to have control samples of the system.

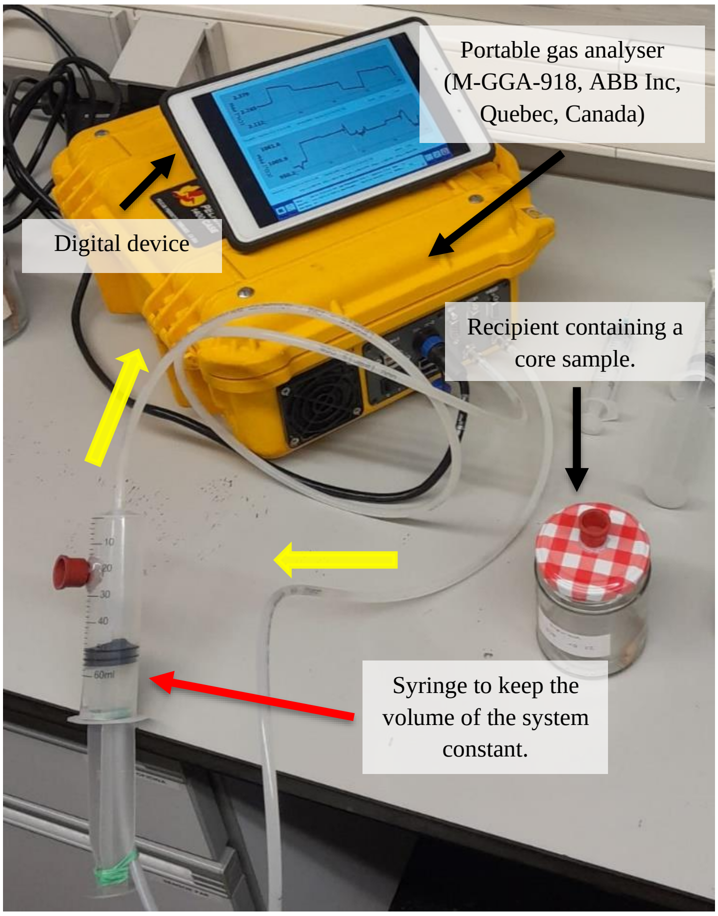


Figure 5. Incubation closed system connected to M-GGA. Gas flux through the gas analyser is marked in yellow arrows. A syringe (red arrow) was used to keep the volume of gas in the system constant.

Incubation data processing

CH_4 and CO_2 concentrations (ppm) of each jar were calculated through the following formula:

$$\text{Concentration} = [(Cf * 101.2) - (Co * 91.2)]/10$$

where *Cf* (ppm) is the concentration of the system after the gas injection; *Co* (ppm) is the concentration of the system before the gas injection; *91.2* (mL) is the volume of the system; *101.2* (mL) is the volume of the system and the gas injection; *10* (mL) is the volume of the gas injection.

Because of the 10mL N_2 injection into the incubation jar, the concentration measures after 48h were diluted. In order to correct this dilution, we calculated the concentration after 24h with the dilution, thus we could adjust the difference between 24h and 48h. This difference is added to the real 24h concentration to determine the 48h concentration.

Using the 3 values of concentration (t=0h, t=24h, t=48h), we could establish an increment of concentration through time. The slope of this increment was used to assess the production of the core. This increment had to be a straight line from t=0 to t=48 to consider a constant production through the incubation period. Nonetheless, this constant production calculated for CH_4 was only evident for the first 24h of the incubation. However, since the CO_2 production held constant during all the 48 h (R^2 was 0.92 on average), we considered that the incubation experiment was properly conducted. Therefore, to determine the CH_4 slope (production), we only used the first two measurements (t=0h, t=24h) as done in other studies (Barba et al., 2021; Pangala et al., 2017). Finally, production was expressed per day and per core dry weight (ppm g^{-1} day^{-1}).

A generalized linear mixed model (GLMM) was used to assess the effect of the treatment (peeled and control) and the tissue type (heartwood, sapwood and cork) on the production. The package *lme4* was used to create the mixed model (Bates et al., 2015). Using the g*lmer* function, tissue and treatment variables were treated as fixed effects, as well as DBH, whereas tree identity was entered as random effect to account for pseudoreplication. The plot variable was treated as a random factor, like in fluxes experiment. Because it was not significant, it was not added to the model. Moreover, we check for interaction between treatment and tissue variables. In order to do it, we only considered two levels in the tissue group, heartwood and sapwood (excluding cork) since cork was not a level in treated trees. This interaction was not significant, therefore the final model did not consider it. The data distribution of the model was family *gamma* with *log* link. The post-hoc comparisons were operated using the *emmeans* function of the *emmeans* package (Lenth, 2024).

RESULTS

Emissions

Cork oaks are capable of emitting methane through the stem, regardless of any variable considered. Moreover, height has an important effect, with emissions decreasing with the stem height, establishing a vertical pattern on tree emissions.

Neither the treatment nor the campaign were significant, nor as single variables or interacting with stem height. Regarding the treatment, the extraction of the cork did not have an effect. Fluxes did not differ between campaigns, showing a lack of seasonality on the fluxes [Figure 6]. Neither soil temperature nor VWC presented a significant effect on stem CH_4 fluxes. Height has an important effect, with emissions decreasing with the stem height, establishing a vertical pattern on tree emissions.

Table 1. Summary table of the fluxes model. Significant differences are symbolized by an asterisk (*), and marginal significant differences by a dot (·). The intercept refers to control trees measured at the bottom part during the first campaign (Campaign 1). The mean error is expressed as standard error (SE). The model has a marginal R^2 of 0.45 and a conditional R^2 of 0.71 (45% of the model is explained by the fixed effects).

	ESTIMATES	**SE**	**P-VALUES**
Intercept	-0.074	0.802	0.926
Treatment (*Peeled*)	0.112	0.152	0.464
Height (*Upper*)	-0.602	0.093	4.12×10^{-10}***
Diameter	0.033	0.017	0.056·
Initial flux	0.016	0.002	$< 2 \times 10^{-16}$***
Soil temperature	0.018	0.031	0.557
VWC	0.013	0.144	0.930
Campaign 2	0.048	0.124	0.702
Campaign 3	0.018	0.131	0.892
Campaign 4	-0.133	0.158	0.399
Interactions			
Soil temperature : VWC	0.001	0.007	0.942
Height (*Upper*) : Campaign2	0.085	0.131	0.514
Height (*Upper*) : Campaign3	0.091	0.132	0.491
Height (*Upper*) : Campaign4	0.048	0.133	0.716
Treatment (*Peeled*) : Campaign2	-0.132	0.133	0.322
Treatment (*Peeled*) : Campaign3	-0.080	0.135	0.552
Treatment (*Peeled*) : Campaign4	0.236	0.136	0.083·

The methane flux of each tree was affected by the individual measured, since the stem diameter was marginal significant with bigger trees that seemed to emit more methane. Moreover, the significance of the initial flux variable indicated that each measure maintained a pattern during the different campaigns of the study.

Interaction between treatment and campaign suggested a temporal effect on the treated trees [Table 1]. However, post-hoc comparisons showed no difference between campaigns while taking into account the interaction with treatment, thus the cork extraction did not affect the methane fluxes. For all the campaigns, fluxes from bottom locations were higher than upper ones [Figure 6], in spite of the treatment. On average, the methane flux from *Quercus suber* was 16.62 nmol m^{-2} s^{-1} (59.83 µmol m^{-2} h^{-1}) ranging from -2.40 nmol m^{-2} s^{-1} to 2439.32 nmol m^{-2} s^{-1}. Less than 4% of the flux measurements were negative (16 measurements).

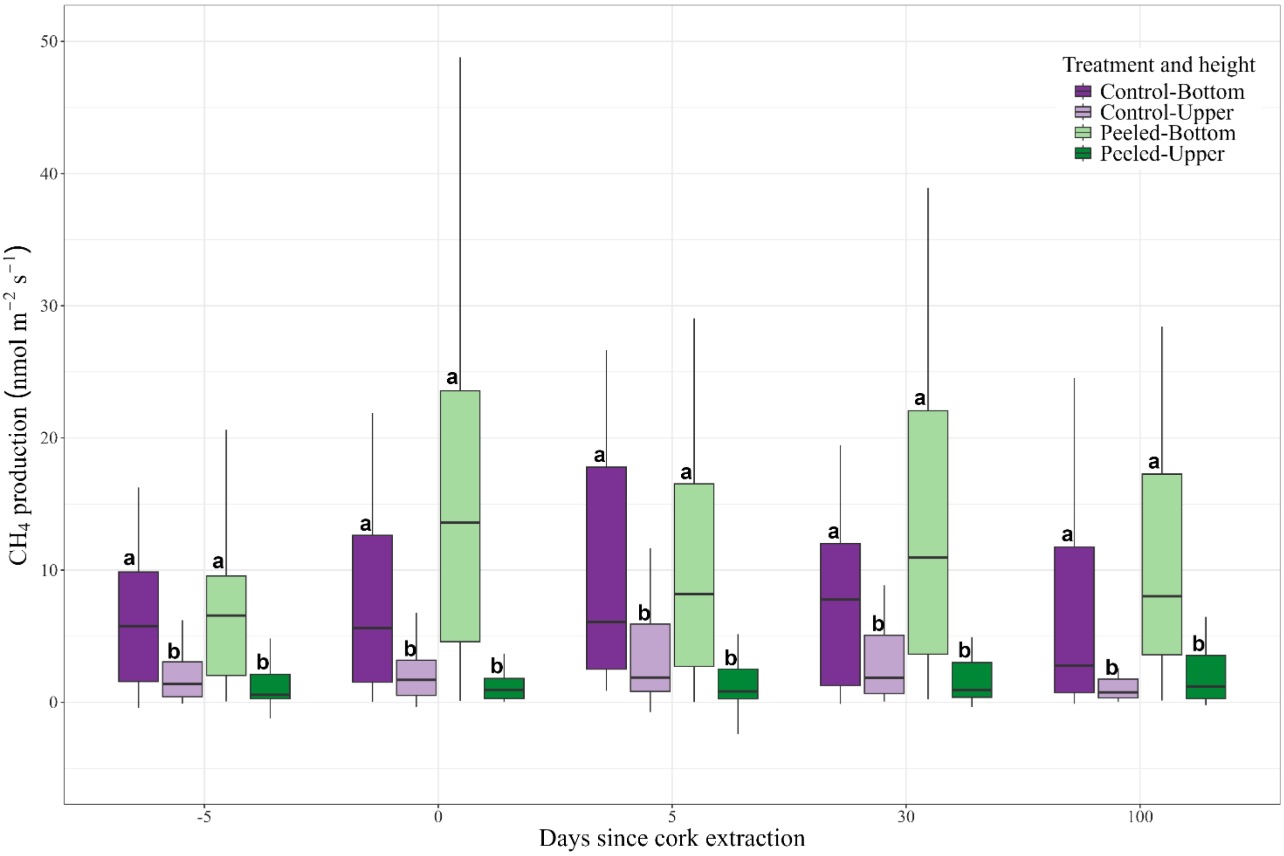


Figure 6. CH_4 fluxes during the 5 campaigns. Colour pattern is used to differentiate the treatment and the measured height. The x axis expresses the different campaigns in days since the cork layer extraction, which was performed on the same day as the second campaign, thus ‘day 0’. Different bold letters indicate significant differences between groups within and across campaigns. The median of each group is expressed by the horizontal black line of the box. Lower and upper hinges indicate the first and third quartiles while upper and lower whiskers indicate 95% confidence interval. Axis were upper limited to 50 nmol m^{-2} s^{-1} for better visualization of the plot.

Incubations

Methane was produced by tree core samples during the incubation experiments, thus cork oak tissues have capacity of CH_4 production. SW and HW tissues presented higher CH_4 production rates than cork tissue [Table 2]. Internal tissues (HW and SW) did not show significant production rates between each.

Moreover, there were no significant differences in CH_4 production capacity between the different treatments, meaning that peeled and control trees might have the same production capacity [Figure 7]. Tree diameter also did not affect the CH_4 production.

Table 2. Summary output of the *glmer* model and post-hoc comparisons of tree core methane production. The intercept refers to cork samples of control treatment, being itself different from 0. Therefore, the values of HW (heartwood) and SW (sapwood) treatments are the comparisons between cork, being all control treatment. Treatment (Peeled trees) row enables comparison between treatments. The mean error is expressed as standard error (SE). The post-hoc results (using *emmeans* function) are expressed as a mean of treatment and peeled of each tissue. Confidence level used is 0.95. The p-value adjustment is done by the *Tukey* method. Significant differences are symbolized by an asterisk (*). The model has a marginal R^2 of 0.11 and a conditional R^2 of 0.43 (11% of the model is explained by the fixed effects).

	ESTIMATES	SE	P-VALUES
Intercept (*Control-CORK*)	-7.342	1.22	1.75 x 10-9***
Tissue (*HW*)	0.921	0.322	0.004**
Tissue (*SW*)	0.722	0.283	0.012*
Treatment (*Peeled*)	-0.064	0.384	0.868
Diameter	0.031	0.043	0.480
Tissue post-hoc			
Cork - HW	-0.921	0.322	0.0116*
Cork - SW	-0.722	0.283	0.029*
HW - SW	0.199	0.244	0.693

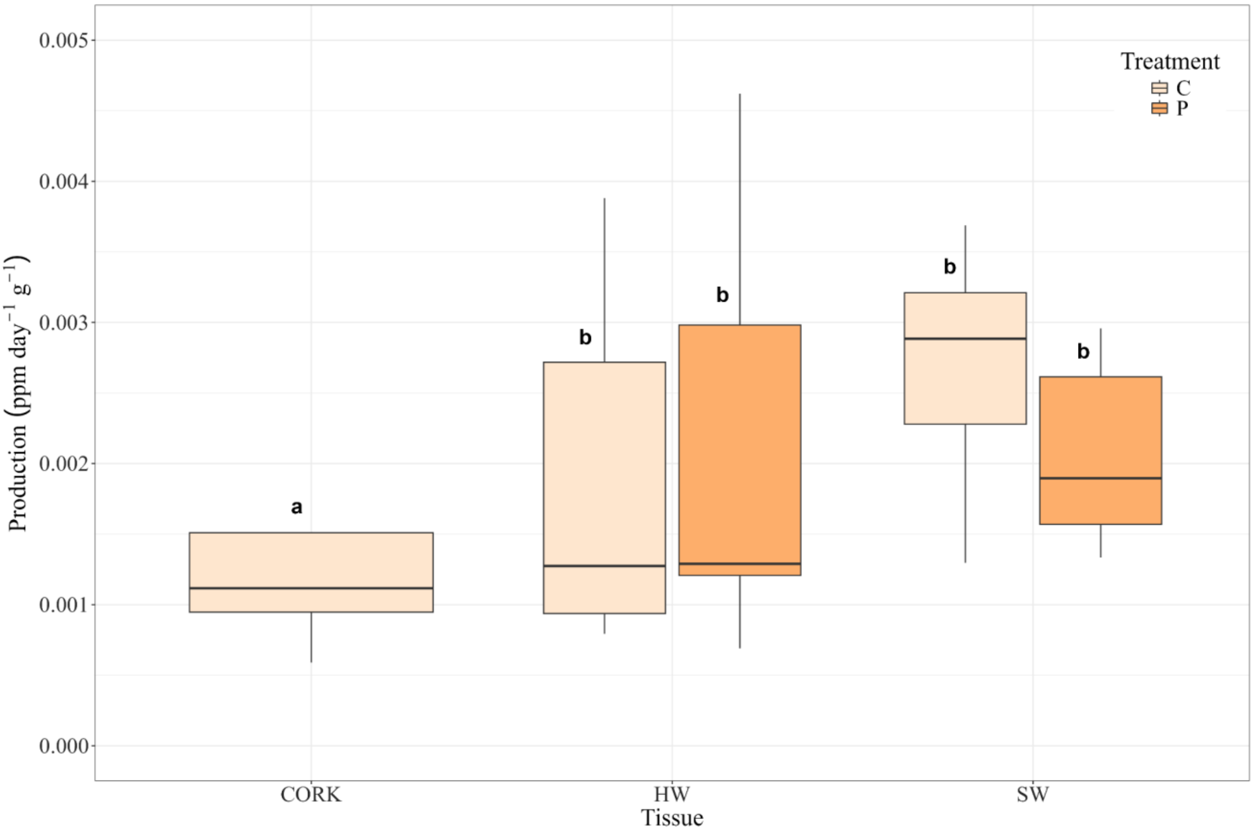


Figure 7. CH_4 production of tree core in ppm day^{-1} g^{-1}. Color pattern is used to differentiate the treatment, where *C* are control trees and *P* are peeled trees. The x axis expresses the different tissue types: cork (CORK), heartwood (HW) and sapwood (SW). The CORK variable does not have P treatment since peeled trees do not have cork tissue. Different bold letters denote significant differences between groups. The median of each group is expressed by the horizontal black line of the box. Lower and upper hinges indicate the first and third quartiles while, that is the 25th and 75th percentiles. Upper and lower whiskers indicate Confidence interval (95%).

DISCUSSION

Mean CH_4 emissions rates during all this study were 59.83 µmol m^{-2} h^{-1}, being similar to emissions measured in temperate climates in recent studies (Maier et al., 2018; Pitz et al., 2018). Hence, cork oak derived methane fluxes in Mediterranean forests have the same magnitude as tree derived methane fluxes studied in upland forests. Nevertheless, higher emissions have been observed on tropical floodplains on angiosperm species, on wetland forests (Pangala et al., 2017). However, wetland tree methane emissions come from soil sources, hence the fluxes measured in wetland tend to be higher than from upland ecosystems (Covey & Megonigal, 2019).

To our knowledge, this study of methane fluxes and production from upland trees is the first study performed in a Mediterranean forest (Covey & Megonigal, 2019). In upland forests, reported methane emissions usually range between 0 and 20.72 $µm^{-2}$ h^{-1} (Covey & Megonigal, 2019), despite some studies found higher emission rates (68.8 µmol m^{-2} h^{-1}), always in temperate climates (Pitz et al., 2018; Wang et al., 2016). Therefore, emissions from cork oak in this study are higher than emissions usually found in upland forests.

The variability of stem fluxes was very high, since the Coefficient of Variation (CV) was 725.16%. In addition, some of the fluxes were above 540 µmol m^{-2} h^{-1}, being 8780 µmol m^{-2} h^{-1} the highest flux. Thus, there was an important tree-specific effect on the tree derived methane flux as indicated by the random factor of the model (tree identity), which explained about 26% of the fluxes variability (difference between marginal and conditional R^2). Additionally, fluxes from the first campaign (one week before peeling) were positively correlated with stem fluxes after peeling, which means there was a consistency in stem fluxes associated with tree identity [Table 1]. This data was supported by other studies where CH_4 fluxes differ from species, as well as individual trees (Pitz et al., 2018).

Although tree diameter at breast height (DBH) was equally balanced between peeled and control trees, it varied among the individuals (between 17.5 cm and 41.1 cm). Some studies have found positive relation between DBH and CH_4 emissions (Pitz et al 2018), despite this relation was not always evident (Warner et al 2017). Wang et al. (2017) found positive correlation between heartwood thickness and stem CH_4 emissions. In our study, DBH was marginally significant ($p=0.0566$) with a positive effect on CH_4 flux [Table 1]. Therefore, DBH contributes to the individual differences between tree fluxes. Nonetheless, these differences are mostly due to the tree itself, since the initial flux is different, and the model is strongly influenced by the random factor [Table 1].

Cork is impermeable to liquids and gasses, such as CH_4 (Gil, 2009), and thus, physical characteristics of the cork material suggested that it could potentially act as physical barrier for methane to diffuse. However, we did not see any difference between control and peeled trees [Figure 6], not even with the second campaign measurements, performed within the first 5 minutes after the cork removal. Therefore, cork oaks can exchange methane with the atmosphere without a major resistance from the cork. Moreover, there were no differences in heartwood and sapwood CH_4 production rates between peeled and control trees, suggesting that cork layer extraction did not affect the methane production either [Figure 7].

Quercus suber stem fluxes had a very strong vertical pattern regardless of the treatment or the temporal dynamics [Table 1]. Fluxes rates were higher at the bottom of the tree, in all campaigns and for both, peeled and control trees [Figure 6]. Vertical pattern in methane fluxes seemed to indicate a soil origin of the emitted gas as found in other studies (Pangala et al., 2014; Pitz & Megonigal, 2017; Terazawa et al., 2007). This gas could be transported from soils through the stem and emitted by degasification, thus, emissions at the bottom part may be higher due to the proximity to its source (Covey & Megonigal, 2019).

In upland forests, some studies have found correlation between stem CH_4 fluxes and soil moisture, suggesting that methane could be produced in the soil under anoxic conditions and transported through the tree (Machacova et al., 2016; Maier et al., 2018). Even in Mediterranean forests, high soil CH_4 emissions could be measured when the water table is close to the topsoil, since this causes more methane production due to anaerobic conditions (Rizzo et al., 2015). However, our study was performed during a drought period [Figure 1] with very low soil water content. Under these conditions, soils on upland forests usually act as net CH_4 sinks rather than sources (Conrad, 2009; Feng et al., 2022; Megonigal & Guenther, 2008). Hence, if CH_4 fluxes in our study were a result of a soil production, we would expect to obtain lower rates (if any). However, our methane fluxes were almost equal as others measured in wetland forests (Gauci et al., 2010), suggesting that methane fluxes in our study might not be produced in soil but inside the trees.

In our anaerobic core incubations, we found CH_4 production in all the tissue layers (heartwood, sapwood and cork) despite the applied treatment [Figure 7]. Therefore, this is solid evidence that the methane source of the trees in our study might be inside the trees. Moreover, production in heartwood and sapwood (internal layers) were significantly higher than the ones in the cork [Table 2]. Internal layers were found to produce CH_4 in the other studies (Wang et al., 2016, 2017), where there was higher concentration of methanogenic microbials (Feng et al., 2022; Yip et al., 2019).

Methane production in trees is one of the main sources of stem fluxes of the gas (Barba et al., 2019; Covey & Megonigal, 2019). This production occurs mainly in the internal tissues, such as heartwood or sapwood (Feng et al., 2022; Wang et al., 2016). Moreover, some studies have found methanogenic communities in these tissues, supporting our theory of internal tree production (Yip et al., 2019). Methane production with the presence of methanogenic communities in the same study have also been seen (Feng et al., 2022). Furthermore, trees in upland forests have the potential to produce methane (Wang et al., 2016, 2017, 2021).

A parallel study performed in the same cork oak trees, studied the microbial communities associated with the CH_4 production and consumption in cork oak tissues (Trullols, 2024). In the inner tree tissues (heartwood and sapwood), he found presence of methanogenic and methanotrophic communities, with more abundance of methanotrophs, despite there were no differences between the two tissue types sampled. Presence of methanogens in heartwood and sapwood strongly support our hypothesis of tree-derived CH_4 emissions produced by tree tissues.

In our study, we found stem fluxes positively correlated with DBH. Even though some studies have found positive correlation between heartwood diameter and stem CH_4 emissions (Wang et al., 2017), other studies suggest that overall DBH is negatively correlated with methanogen abundance (Yip et al., 2019), hence production should decrease with DBH. Trees with higher DBH have higher heartwood diameter, and heartwood diameter is positively correlated to tree volume (Miranda et al., 2015). Relation of wood volume and stem area is higher in larger trees, suggesting that higher production rates could be found in bigger trees.

The vertical pattern of methane fluxes shown in this study may be not related to soil origin of CH_4 but to different cork physical properties between bottom and upper position. Even though the bottom part of the control cork oaks had not been peeled for the last 12-14 years, they have been peeled before, at least once (Q. Gubau, personal communication). When the cork layer is extracted, it starts growing again 25-35 days after the peeling (Pereira, 2011). After 10 years of the extraction, the new cork layer is thinner than the original one and can be fractured (Pereira, 2011). Hence, the bottom part of all the trees in our study may be thinner, more fractured, and therefore, less impermeable, than the upper part. Thus, the CH_4 flux may be significantly higher at the bottom part, masking the effect of the treatment. This assumption is supported by our results since the difference between bottom and upper measurements are already seen in the first campaign, regardless of the treatment effect (extraction of the cork at the second campaign, one week later). Moreover, the growth of the cork layer is limited by drought conditions (Pereira, 2011). The Mediterranean climate is characterized by dry summers (Lionello et al., 2006), thus the cork layer growth of the trees in our study may be limited.

CONCLUSIONS

The main objective of this study was to quantify the magnitude of the CH_4 stem emissions of *Quercus suber* and to determine the potential effect of cork removal on those emissions. Our experiment, the first one performed in Mediterranean species, demonstrates that cork oak emits methane through the stems. Moreover, the fluxes of this species are one of the highest found in upland forests (59.83 µmol m^{-2} h^{-1} on average). No differences were found between treatment and control, thus the extraction of cork layer did not have an effect on CH_4 fluxes. Laboratory incubations of tree cores under anaerobic atmosphere showed a strong capacity of CH_4 production for all measured trees, suggesting that CH_4 was produced within the tree rather than produced in the soil and transported through the roots and the stems. The internal tissues (heartwood and sapwood) have greater capacity of production than the cork layer, and the treatment did have no effect on the production. Moreover, parallel to our study during the same period, a study found presence of methanogens inside the same trees we measured, reinforcing our results (Trullols, 2024).

We found a height pattern of the CH_4 fluxes within the trees with methane emissions being higher at the bottom of the tree than upper in the stem. However, this pattern was not caused by the extraction of the cork since there was no significant difference between the peeled trees and the control trees, or between before and after peeling for the same trees. Higher emissions at the bottom usually suggest that the soil is the source of the emitted CH_4 (Machacova et al., 2016; Maier et al., 2018), but the lack of correlation with soil moisture or soil temperature, and the positive production of tree cores pointed towards internal production.

We suggest that the height pattern was due to the characteristics of the cork layer on the bottom part. Since all of the trees in the study have been peeled at least once, all the bottom parts may be thinner and more fractured than the upper ones (Pereira, 2011). Hence, methane exchange from the tree to the atmosphere may be less limited in the bottom parts, making emissions decrease with height.

More studies of tree-stem derived CH_4 emissions on Mediterranean climate species are needed in order to assess if the results of this study are due to the characteristics of cork oak or are globally found in species with similar climate conditions.

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