Reproduction of methane emissions from terrestrial plants under aerobic conditions

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Abstract

Latest research confirmed that plant matter can also produce a non negligible amount of the greenhouse gas methane under aerobic conditions. These findings have led to discussions and controversy within the research community. While KEPPLER ET AL. (2006) as one of the first researchers published results supporting this hypothesis others like DUECK ET AL. (2007) refuted these results within their experiments.

In this study we demonstrate – using experimental techniques partly based on Kepplers study from 2006 – that detached and air dried organic matter emits methane under aerobic conditions. Plants were incubated under laboratory conditions while the data was collected first under light followed by dark conditions. Released methane was found for all 20 investigated species in a range of 1.7 to 25.8ppm under light conditions (mean value) and in a range of 1.9 to 28.8ppm under dark conditions (mean value). Significant amounts occurred for coniferous wood including spruce and fir. Further high methane concentrations could be observed for the specie bux.

We assume that these findings have an important influence on the previous suggestions concerning the global distribution of methane sources.

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Introduction

Next to carbon dioxide methane is a very important greenhouse gas and its influence on atmospheric chemistry is about 21 times higher. Methane originates from human-related and natural sources. According to IPCC (2001) estimations 60% of global methane is emitted by anthropogenic sources including fossil fuels, food production (rice, cattle) and biomass burning. Main natural sources are wetlands. Summed up all these major sources lead to a global budget of ~600Tg yr^{-1}.

According to established knowledge methane is produced by bacterial activity under anaerobic conditions. Recent investigations indicated that in the tropical regions above evergreen forests an additional source of 30 – 40Tg methane exists (time period August-November). These observations led to further research (KEPPLER ET AL. 2006).

KEPPLER ET AL. (2006) who presented their results as a first estimate, observed in their experimental setup methane release from all living and detached plant materials under aerobic conditions. In their studies methane release is mainly dependent on temperature. Between a range of 30-70°C methane release doubled with every 10°C increase. Following studies of VIGANO ET AL. (2008) and MCLEOD ET AL. (2008) described a linear relation between aerobic methane release and UV radiation.

Also WANG ET AL. (2008) investigated methane emissions under aerobic conditions. Their experiment included 44 species in the Inner Mongolia Steppe. Under dark conditions over 80% of their plants did not produce any measureable methane. The other 20% only produced soil-derived methane in stem tissues. Only leaves of *Artemisia frigida*, a xerophytic shrub, produced a measureable amount of methane (KIRSCHBAUM ET AL. 2008).

In contrast to those findings DUECK ET AL. (2007) and BEERLING ET AL. (2008) tried to improve Kepplers experiment without finding any significant methane emissions. BEERLING ET AL. (2008) used two different plants (*Zea Mays* and *Nicotiana tabacum*) and analysed their reaction towards changes in light and dark and constant temperature conditions (25°C). DUECK ET AL. (2007) worked with a stable isotope ^{13}C and a laser-based measuring technique. In none of their six investigated species any aerobic methane emission was detected. Their findings highly differed from earlier studies. They could not reproduce Kepplers experimental results because their plants were grown up in greenhouses under artificial light without UV radiation.

In further experiments Keppler investigated how methane is produced under UV light. Based on established knowledge that pectin contains methoxy groups in which the chemical methane structure is partly recognizable they used an isotope analysis: Pectin was labelled by replacing the hydrogen atom within the methoxy groups with deuterium. Afterwards the deuterium was found in the emitted methane. But also methane without deuterium could be found whose source has not been defined yet (KEPPLER 2008, MAX PLANCK INSTITUTE).

Materials and Methods

The whole experiment is based on KEPPLER ET AL. (2006) study "methane emissions from terrestrial plants under aerobic conditions" published in Nature.

KEPPLER ET AL. (2006) used fresh collected and dried plants from 20 different plant species including trees, grasses, C3- and C4-plants (vary in different way of carbon fixation during photosynthesis)

In the first part of his experiment detached leaves were incubated. Fresh leaves (1-6g detached from intact plants) and dried leaves (1-5g air dried at 25°C for 48 hours) were put into glass vials with 44ml volume and closed with silicon septa. Before the start of the experiment the used vials were cleaned with methane-free air for one hour. To determine the incoming methane due to leaking control blanks were used and measured after purging. First the filled vials were incubated in the dark for 16 hours at 30°C and 40°C. Afterwards the methane was analysed by an isotope ratio mass spectrometry (CF-IRMS). To investigate methane emissions related to solar radiation the vials were put into direct sunlight for one hour between 10:00h and 15:00h in Heidelberg, Germany. While direct sunshine experiments took place in spring (March-May 2005) the dark experiments were carried out in laboratories.

By drying the material for 24 hours at 105°C the leaf dry matter was defined.

The next step of the experiment contained plexiglass chambers with a volume of 18l and a diameter of 29cm. The living plants were put into closed but air circulated chambers and purged with methane-free air till the methane level within the chamber was below 10ppb. All influencing parameters (e.g. temperature, humidity) were monitored during the whole incubation time. The CO_2 concentration was always kept above 300ppm. Methane concentrations as well as $\delta^{13}C$ (stable carbon isotope) were determined every 25 minutes by taking a sample of 40ml out of the chamber headspace.

As in the first part of the experiment the leaf dry matter was measured afterwards and also plexiglass chambers were put into direct sunlight for 20 minutes between 10:00h and 15:00h in Heidelberg, Germany.

For analysing the methane concentrations the CF-IRMS was used after separating its components by the gas chromatograph (GC).

The calculation of the annual methane production is based on daily emission rates under consideration of measured methane emissions with and without direct sunshine and net primary production (KEPPLER ET AL. 2006).

Experimental Setup

The idea of this project was to replicate the experiments conducted by Keppler in 2006. Due to the availability of technical devices and different circumstances like growing season and time the experimental setup was modified.

Before starting the experiment we purged 27 vials two times with methane-free air (zero-gas: nitrogen and oxygen) to determine the amount of leakage. We used glass vials with a volume of 100ml which were sealed with a plastic lid containing a rubber for injections. 48 hours after purging we took samples of 10ml out of each vial to analyse the methane concentration with the gas chromatograph (GC). For our experiment we used the 21 vials with lowest leakage. 20 vials were filled with 1-5g of detached leaves which were dried 48 hours at 29°C before. From the beginning of the measurements one vial was used as a control-blank. Additionally six more control blanks were used since 4th of December (beginning of dark measurement) to get more replicates and greater validity. To observe possible reactions of the vial material with its content, the blanks were filled with different matters. Four were used dry, two wet (5ml water) and one with a mixture of water and organic material (fir needles). After filling the vials they were purged again two times with methane-free air.

Our experiment is based on 20 species, while a few species differ from Kepplers used plants, because of seasonally availability of plant material. The investigated plants represent two different categories. C4-plants: red sorghum, sorghum and sorghum seeds. C3-plants (temperate regions): beech, maple, basil, rosemary, spruce, perennial ryegras, sweet vernal grass, fir, bux, kaukasic wingsnut. C3-plants (tropical regions): banana, spanish moss, sword fern, crane flower, lily, lemon and cocoa.

The following table gives an overview on the investigated plant material divided into origin and way of carbon fixation. Also given is the moisture content before the first incubation. Collected leaves were weighed before and after drying at 29°C for 48 hours.

Table 1: species overview, origin and moisture content

species	origin	moisture content before incubation (%)
C3-leaf (temperate regions)		
basil *(Ocimum basilicum)*	commercially available	90.67
beech *(Fagus sylvatica)*	collected in Lund	49.0
bux *(Buxus mycrophylla)*	collected at the Botanical Garden Lund	36.42
fir *(Abies nordmanniana)*	collected at the Botanical Garden Lund	38.0
kaukasic wingsnut *(Pterocarya fraxinifolia)*	collected at the Botanical Garden Lund	56.84
maple *(Acer platanoides)*	collected in Lund	51.69

perennial ryegrass *(Lolium perenne)*	collected in Lomma	29.07
rosemary *(Rosmarinus officinalis)*	commercially available	71.33
spruce *(Picea abies)*	collected at the Botanical Garden Lund	38.41
sweet vernal grass *(Anthoxanthum odoratum L.)*	collected in Lomma	0
C3-leaf (tropical regions)		
banana *(Musa textilis)*	collected at the Botanical Garden Lund	82.26
cocoa *(Theobroma cacao)*	collected at the Botanical Garden Lund	4.08
crane flower *(Strelitzia reginae)*	collected at the Botanical Garden Lund	27.42
lemon *(Citrus auratifolia)*	commercially available	28.00
lily *(Lilium candidum)*	commercially available	57.65
spanish moss *(Tillandsia usneoides)*	collected at the Botanical Garden Lund	8.70
sword fern *(Nephrolepis exaltata)*	commercially available	79.33
C4-leaf		
red-sorghum *(Setaria italica)*	commercially available	6.67
sorghum *(Setaria italica)*	commercially available	16.67
sorghum seeds *(Setaria italica)*	commercially available	4.29

First the vials with the detached plant materials were randomly incubated in an incubation chamber at 35°C with light for 16 hours. Afterwards 10ml of methane free air were injected into the vial by a syringe and a 10ml sample out of the vial was analysed by the GC. After five to seven samples we ran at least three standards (std). The standard gas consists of 600ppm carbon dioxide and 12ppm methane.

Altogether the vials were incubated six times under constant conditions, while the samples were taken after different time periods (16 - 96h).

For the incubation in the dark we switched off the light of the incubation chamber. Other parameters like temperature were not changed. Again we measured 6 times after different time periods (24 – 120h).

To investigate possible temperature increases/decreases because of light influence, parallel temperature measurements within the chamber were conducted. For continuous temperature measurements of ten minutes intervals the iButton-TMEX Runtime Environment was used. Altogether three temperature sensor buttons were put into three vials of 500ml size, filled with organic matter to ensure similar conditions. We incubated two vials on the right and left side and one in the middle of the chamber.

Calculation of methane emissions

All used equations for the calculations are mainly based on the Ideal Gas Equation and modified or adjusted concerning our experimental setup.

The first step of the calculations is to control the standard input for further equations. Therefore the average is calculated of the standards of one measuring day.

Afterwards the standard deviation (T) for these standards was determined. For further equations only standards within +/-3*T were used. The example in figure 1 shows that in this case one standard (number of std 9) had to be excluded.
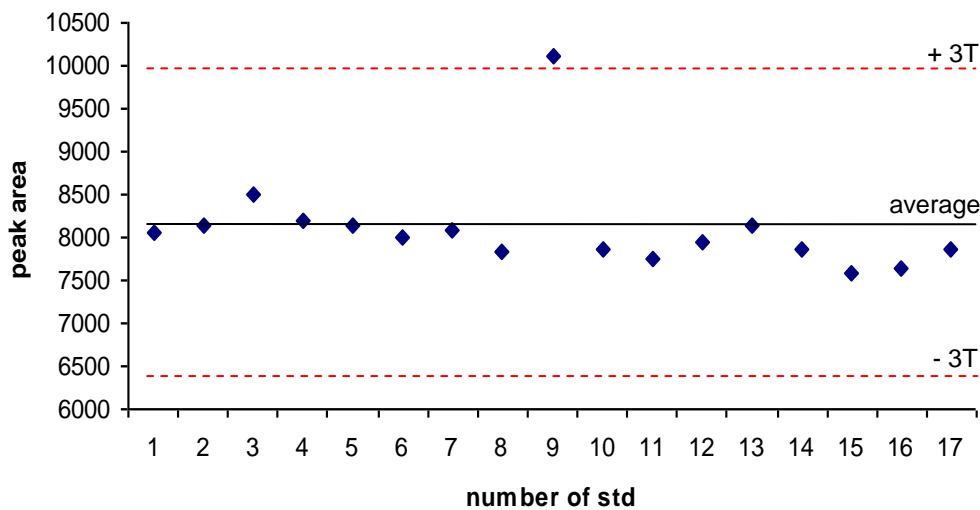
Figure 1: Control card

The measured ppm values of the GC are not always reliable. To avoid possible failing outcomes the concentration numbers had to be corrected.
The calculation includes the daily mean of the standard peak areas, the known methane concentration of the standard gas (12ppm) and the peak area of the sample.

$$\textbf{12ppm * peak area}_{\textbf{sample}} \textbf{ / mean of the standard peak areas}$$

To include a dilution factor the methane volume (ml) before and after sampling was determined.

Before dilution:

$$\textbf{100ml (vial volume) * corrected concentration (ppm) / 1 000 000}$$

After dilution:

$$\textbf{110ml (vial volume after dilution) * corrected concentration (ppm) / 1 000 000}$$

In the next step the methane volume (ml) was converted to methane mass (mg). Therefore the molecular mass of methane (16g/mol) was multiplied with the methane

volume before and after dilution and divided by the methane molar volume at 35°C (308.15K) and pressure 1 atmosphere.

The methane molar volume was calculated as followed:

Molar volume at standard T and P (22.4 l/mol) * T (K) / 273.15K

Together with incubation time and the biomass dry weight (mg), the difference of the methane mass (Δmg) before sampling and after sampling of the measuring day before, were inputs of the final equation to define the methane emission (mg CH_4 mg_{dw}^{-1} h^{-1}).

Δ CH_4 (mg) / incubation time (h) / biomass dry weight (mg)

Results

Figures 2 to 13 show the methane emissions as well as methane concentrations over four weeks at twelve measuring days. The results are shown as methane concentrations (ppm) which describe the actual methane in the vial after dilution. Methane emissions (mg CH_4 $mg_{dw}^{-1}h^{-1}$) present changes like increase and decrease within the vial between the measurements dependent on the dry weight. The samples till 4th of December were taken under light conditions and a chamber temperature of 35°C. In the following period of the experiment, the light was switched off while the temperature conditions were kept constant. The changes in the experimental setup according to light conditions are shown in the figures as a black line between the 3rd and the 4th of December.

The observed results for red sorghum showed the most significant dependence on light conditions within the incubation chamber. During the light period methane concentrations increased from 1.1 to 9.5ppm. With a minimum of 3.87E-07 and a maximum of 1.8E-06mg CH_4 $mg_{dw}^{-1}h^{-1}$ the methane emission rates showed a similar increase (see figure 4 and 5).

The methane concentrations for bux increased from 2.7 to a maximum of 38.7ppm within twelve days (including five measuring days), yielding emission rates in the range -3.9E-07 to 3.07E-06 mg CH_4 $mg_{dw}^{-1}h^{-1}$ (see figure 8 and 9).

Significantly decreased methane concentrations were measured under dark conditions. It is important to note that already one measurement before the dark period slightly decreases in methane concentrations were observed.

The specie fir is showing a similar progression. The highest increase in concentration from 3.6ppm to 38.3ppm is also observed for the first five measurements. Afterwards the concentrations decreased rather continuously (see figure 6 and 7).

Almost the same results were found out for the leaf dry matter of banana, while the concentrations were always below the values of fir. During a period of twelve days the concentrations increased in a range of 19ppm (see figure 12 and 13).

Contrary to most of the other investigated species, the methane release of lemon increased only during the first two measurement days. After a maximum of 14.7ppm the values decreased rather continuously. Lemon showed the less dependence on light and dark conditions (see figure 12 and 13).

In general the concentration of 75% of our selected species (16 of 21) was increasing in the light experiment, while the emission rates were varying. The following decrease of methane concentration within the dark period led to the assumption that there could be sensitivity towards light.

The measuring results of the dry control-blank, which was incubated from the beginning, were almost constant during the whole experiment. Like the other vials also the control-blank showed a decrease in methane concentration on 3rd of

December. The other vials showed, independent of their contents, extensive differences in methane concentrations while the rough progress was similar (see figure 10 and 11).
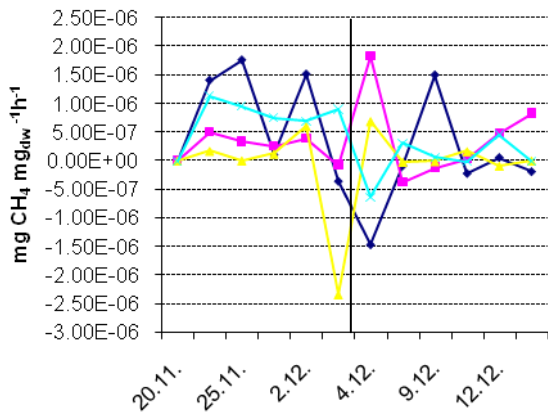

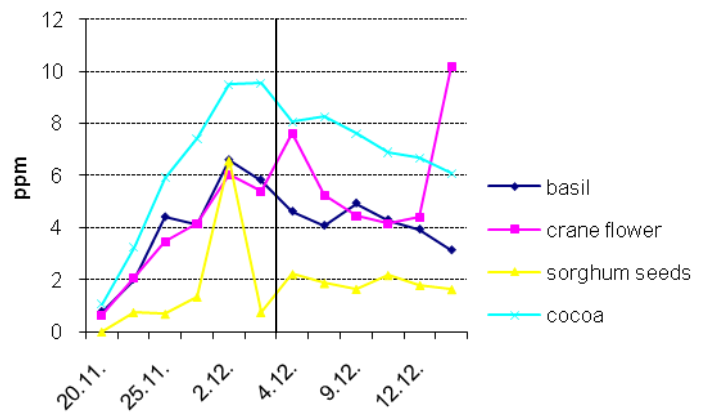**Figure 2: methane emission rates for single samples**

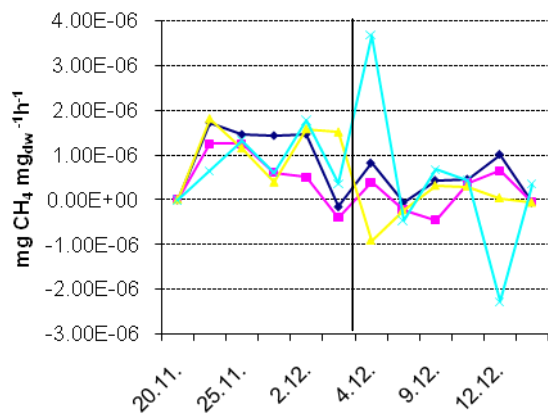**Figure 3: methane concentrations for single samples**

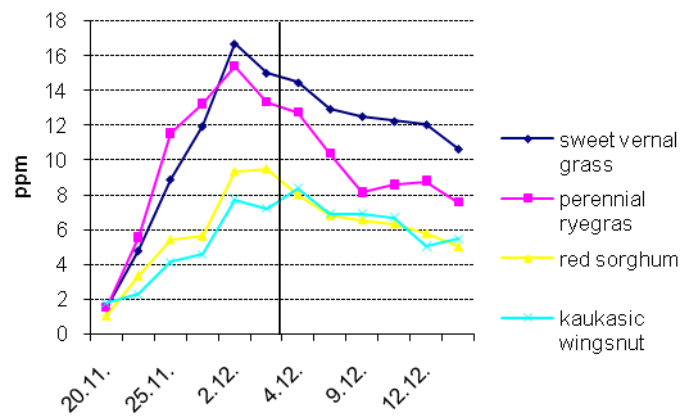**Figure 4: methane emission rates for single samples**
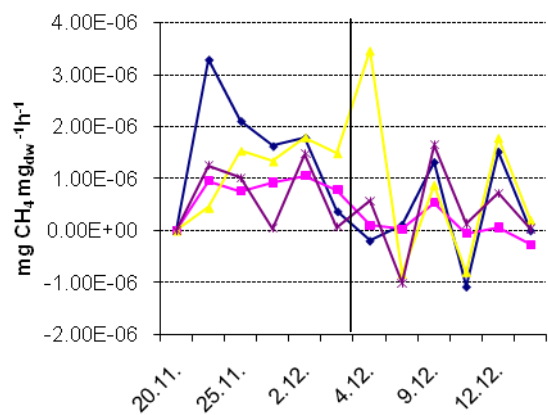

Figure 5: methane concentrations for single samples

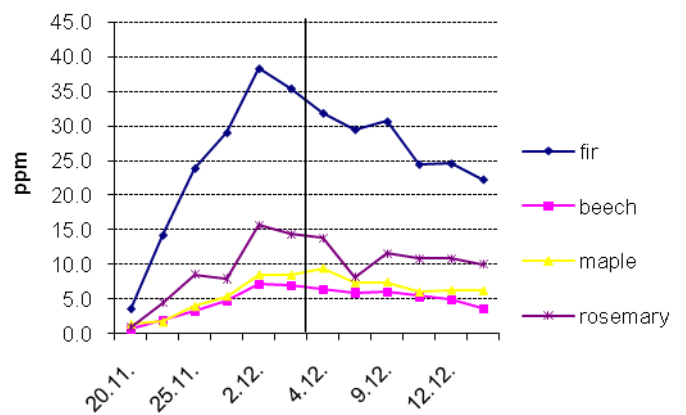

Figure 6: methane emission rates for single samples

Figure 7: methane concentrations for single samples
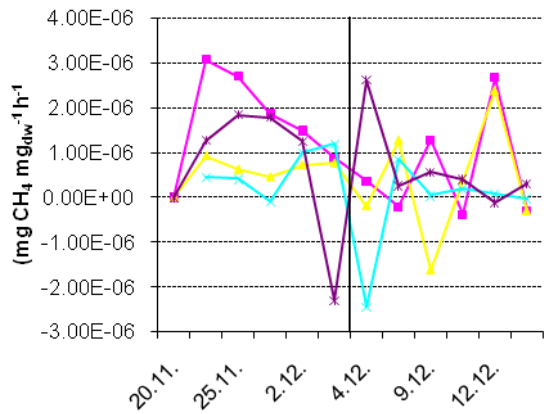
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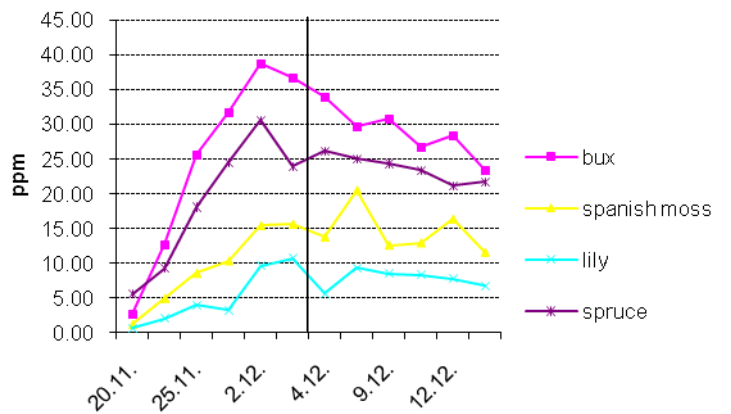**Figure 8: methane emission rates for single samples**

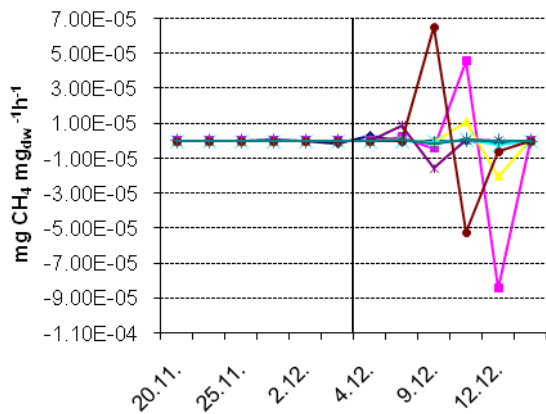**Figure 9: methane concentrations for single samples**

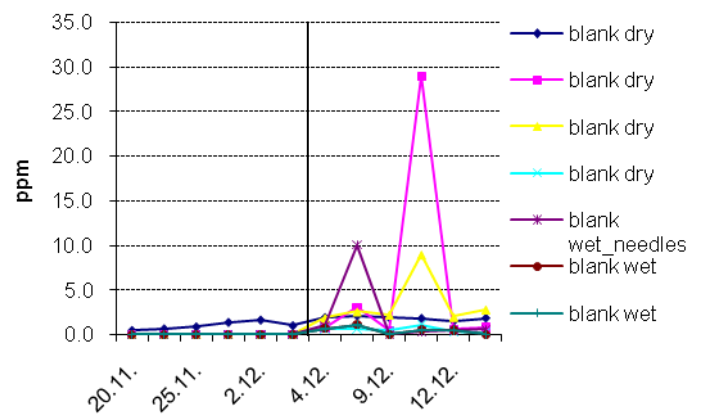**Figure 10: methane emission rates for single samples**
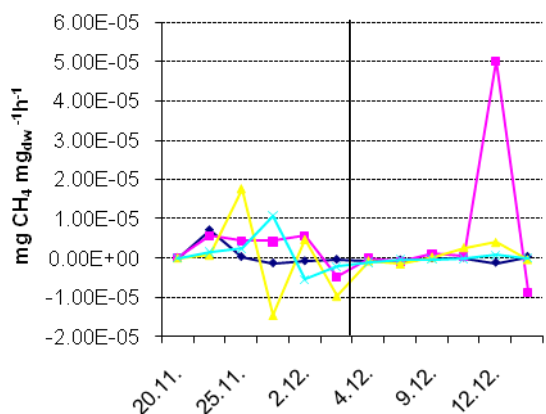

Figure 11: methane concentrations for single samples

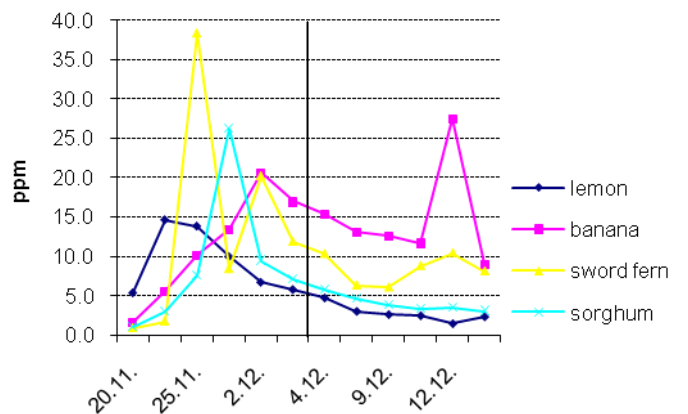**Figure 12: methane emission rates for single samples**

Figure 13: methane concentrations for single samples

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The following table gives an overview on the leaves dry weight, mean methane concentrations and emissions. Different species can be compared under light or dark conditions, while the emissions or concentrations have to be put into relation to the dry weight.

Table 2: Methane emissions and concentrations of investigated plants for light and dark conditions

species	matter dry weight (g)	mean concentration (ppm)		mean emission rates (mg CH4 mgdw -1h-1)	
		light	dark	light	dark
C3-leaf **(temperate regions)**					
basil	1.4	4.0	4.2	8.89E-07	-6.6E-08
beech	2.0	4.1	5.4	9.04E-07	6.98E-08
bux	5.0	25.6	28.8	2E-06	5.65E-07
fir*	5.0	24.1	27.2	1.83E-07	2.72E-07
kaukasic wingsnut	1.5	4.6	6.6	9.45E-07	4.04E-07
maple	1.5	4.9	7.1	1.32E-06	7.81E-07
perennial ryegrass	5.0	10.1	9.4	5.32E-07	1.11E-07
rosemary*	4.3	8.6	10.9	7.65E-07	3.42E-07
spruce*	5.0	18.6	23.6	7.67E-07	6.68E-07
sweet vernal grass	3.0	9.8	12.5	1.18E-06	4.3E-07
C3-leaf **(tropical regions)**					
banana	1.1	11.4	14.9	3.01E-06	7.01E-06
cocoa	3.0	6.1	7.3	8.86E-07	3.02E-08
crane flower	4.5	3.6	6.0	2.76E-07	4.43E-07
lemon	2.0	9.4	2.8	9.24E-07	-3.90E-07
lily*	5.0	5.1	7.8	5.87E-07	-2.2E-07
spanish moss*	6.3	9.4	14.7	6.92E-07	3.12E-07
sword fern*	2.0	13.6	8.4	-2.7E-07	7.06E-07
C4-leaf					
red-sorghum	2.0	5.7	6.4	1.28E-06	-9.7E-08
sorghum	2.0	9.1	4.1	1.4E-06	-1.8E-07
sorghum seeds	6.7	1.7	1.9	-2.9E-07	1.2E-07

*showed moisture after incubation

Discussion

From the beginning of the experiment the external influences which could cause or reduce methane emissions were only partly determined.

The chamber setup monitor showed a constant temperature curve under light and dark conditions. But additional temperature measurements with the iButton-TMEX Runtime Environment confirmed light influence on temperature. We could prove that under light conditions the temperatures within the vials were up to 10°C higher than the chamber monitor has shown. While the temperature changes within the vials under dark conditions were negligible.

During the experiment it was not possible to define the light source in detail. So influence of UV radiation on methane emissions could not be investigated. Because of UV resistance of the vials no further experiments were conducted.

Within the vials also negative emissions occurred what gives reason to the assumption that methane reducing factors must be considered. Leaking of methane in the vials through the lids could be induced by incorrect closing of the flasks. An eroded septa after several measurements could also enhance further leaking. Other possible factors causing a decrease of methane are adsorption processes of dried leaves or methane oxidation within the vial.

First increases in methane concentrations could be traced back to the fact that methane is physically bonded to the leaf surface (adsorption) which is released. But this could be dismissed after the methane concentration within the vials exceeded the atmospheric methane concentration of 1.775ppm. Other authors also refer to release from in plant reservoirs stored methane.

It could not be excluded that the vial material was causing methane emissions. Also possible reasons for increasing emissions could be chemical reactions between the septa and organic material or water.

The observation that all plants independent on their functional types emitted at least a small amount of methane gives reason for the assumption that the emissions are not of biological but chemical origin.

The fact that methane concentrations occurred in each vial could imply that the measured results were not released by the organic matter but caused by chemical reactions of the flask material.

During the whole experimental period the number and quality of control blanks was not reliable. Within the first part (light experiment) only one control blank was used which can not be counted as representative. For the second part six more control blanks were incubated. For these all the rest of the vials with the highest leaking were used.

50% of the investigated plants (lemon, bux, fir, maple, beech, cocoa, basil, sweet vernal grass, perennial ryegrass, red sorghum, kaukasic wingsnut) showed a clearly

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maximum of methane concentrations, independent on light or dark conditions, followed by a constant decline. It could be suspected that these plants reached a saturation point.

The other half showed partly inconsistent increases and decreases with several maxima, independent on the chamber conditions.

Especially spruce and crane flower (Figure 9 and 3 respectively) demonstrated an unnatural decline before switching off the light. The most likely reason for that could be technical or working mistakes. But that could not be proved.

To exclude any mistakes like mentioned above, it would have been useful to have at least two replicates for each species.

Besides, the origin and previous treatments of the leaves (e.g. pesticides) during the growth could influence the chemistry within the vials.

Comparison with Kepplers study

The whole experiment tended to replicate Kepplers study as far as possible. We were forced to differ partly from his setup, because of material availability e.g. vials, septa and lids. Due to not available equipment it was not possible to measure UV radiation and thus further investigations concerning UV influence on methane emissions could not be operated. Further more the fact that the vials are not permeable to UV radiation limited our experimental setup. Investigations according direct sunlight exposure could not be conducted.

In Kepplers study a detailed description of incubation times is not mentioned. That is why we elaborated our own time table including incubation times considering our time frame of five weeks.

Kepplers study was run from May to August. In contrary our experiment took place in November and December what influenced our choice of plants. We did not get wheat, ash, alula, lemon grass, maize and sugar cane. Instead we used bux, lemon, red sorghum, sorghum seeds, fir, kaukasic wingsnut and cocoa to provide a representative number of C3- and C4-plants.

A comparison of specific results is not possible because of too many differences in the experimental setup and missing data of emission rates for single species. In agreement with Keppler we can confirm that all investigated plants emit methane under aerobic conditions.

Conclusion and outlook

Our results concur with KEPPLER ET AL. (2006) that all of the investigated plants emit methane under aerobic conditions and refute DUECK ET AL. (2007) that plants do not emit. In our experiment the leaf dry matter did not show any reaction towards light influence concerning methane production. Furthermore physically adsorption can be excluded, but methane release caused by material like vial and septa could not be clearly disproved.

We recommend the controlled growing of the used plant species to exclude uncertainty factors concerning any kind of chemical treatment.

In further comparison with KEPPLER ET AL. (2006) we will investigate temperature influence on the leaf dry matter. Therefore temperature will be decreased in 10 °C intervals. Keppler showed in his study a very strong temperature sensitivity of the plant material, it would be interesting to see if these observations can be reproduced. In addition we will conduct tests on living plants for a few species.

We did not experiment with different wavelengths of light. For detailed information we refer to the study of VIGANO ET AL. (2008). Their study included UV light influence on methane release from dried and fresh detached leaves.

Experiment 2

Introduction

At the previous experiment the leaf dry matter within the vials were of different decomposition states. The investigated leaves were directly collected from trees as well as collected from the ground. Hence we tested the possible influence of leaf age on the methane emissions. These measurements were not part of Kepplers study.

Material and Methods

For detailed description of the used material we refer to page 6ff. We decided to use three species which were easily commercial available, rosemary, basil and catgrass. While the last mentioned specie was grown up in the laboratory.

At the beginning we filled between 12 and 15.7g of fresh detached leaves into three paper bags for each specie. These bags were dried in different stages; 24 hours, 48 hours and 72 hours at 29°C. To determine the moisture content related to different drying levels we weighed the plant material before and after drying.

Parallel we filled the vials with fresh detached leaves of all three species and incubated them for 24 hours at 35°C (light). Afterwards also the in different stages dried leaves were incubated each for 24 hours and 35°C. For each specie and each drying level two measurements were run.

Methane analysis and flux calculations were conducted as in the first experiment (see p. 6ff).

Results

First it is to note that figures 14 and 15 can not be seen as a flow diagram. Comparable are only the measuring results of the same drying levels. For the drying stages fresh, 24 hours and 72 hours the investigated plants show different progresses. For 48 hours dried leaves, the graphs are similar and have a significant increase in methane concentrations (see figure 15). The maximum of methane release was measured for rosemary after 24 hours drying, for catgrass after 72 hours drying and for basil after 48 hours drying.
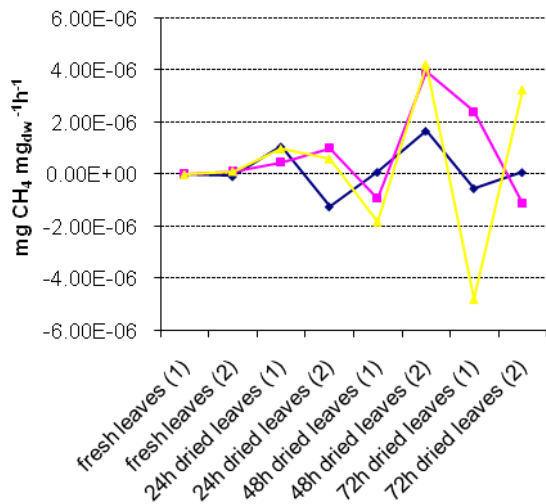
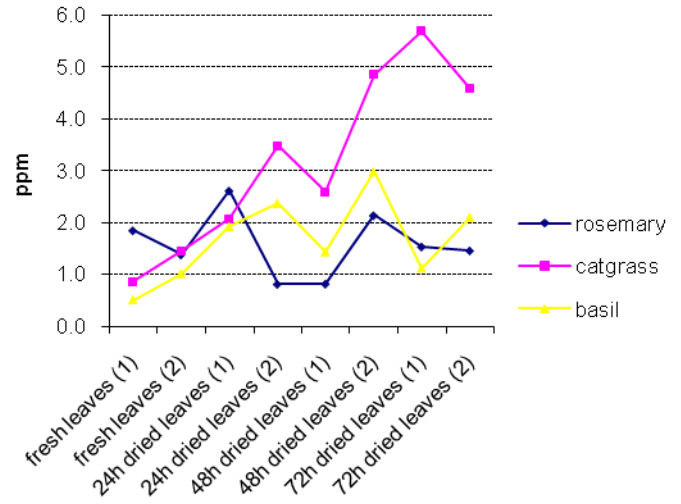**Figure 14: methane emissions of different dry levels**

Figure 15: methane concentrations of different dry levels

Discussion

For the whole experimental setup the same internal and external influences mentioned above (see page 12f) on methane increase or decrease within the vials have to be considered. Concerning the second experiment it is to note that only remaining vials with the highest leaking could be used. That is why the results might be slightly falsified.

Additionally a comparison between the particular drying levels is only acceptable under reserve because on the one hand vials with different qualities were used what implies different leaking rates. On the other hand for each drying level different leaves of one species were incubated.

Conclusion

In general to get more reliable results the collection of data has to be extended at least with one data point per drying level. Depending on the plant physiology and initial moisture content each plant could have its own optimum in methane release.

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