

# Rhizosphere Priming and Methane Oxidation in Agricultural Soil

Effect of Land Use on Atmospheric Concentrations of CO<sub>2</sub> and CH<sub>4</sub> and Climate Change

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**Front page** Photo by Esben Øster Mortensen (26/5-2017) showing the experiemental fields of ley (left) and arable land (right) used in this study.

# Rhizosphere Priming and Methane Oxidation in Agricultural Soil

Effect of Land Use on Atmospheric Concentrations of CO<sub>2</sub> and CH<sub>4</sub> and Climate Change

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# Abstract

Rhizosphere interactions have high influence on the fluxes of carbon to and from terrestrial ecosystems. One very important part of the rhizosphere interactions is the rhizosphere priming effect (RPE) which is defined as changes in the decomposition rate of soil organic matter (SOM) in response to labile organic carbon input from plant roots. Changes in decomposition of SOM affect nutrient availability and nutrient cycling, and does also bring changes in emissions of CO<sub>2</sub> from soil. Therefore, changes in the RPE can have a crucial impact in the ecosystems of soil and on the atmospheric concentrations of CO<sub>2</sub>. Oxidation of methane in soil is another component affecting carbon fluxes in soil and global climate. The aim of this study was to test whether differences in priming could be observed in soil of different agricultural land uses; arable land, pasture and ley, and at the same time measuring methane oxidation to test if any differences occurred between the three different land uses. According to the *microbial mining hypothesis*, it was hypothesized that the highest levels of priming would occur in pasture soil with low availability of nitrogen. Due to expected low concentrations of nitrogen and especially ammonium, methane oxidation was also expected to be highest in pasture. Priming was observed as significant changes in SOM decomposition due to glucose amendments. No significant differences were found between arable land, pasture and ley in terms of SOM respiration, priming or methane oxidation. However, a pattern supporting the *stoichiometric decomposition theory* was observed, as pasture on average had the lowest priming effect. At the same time, the highest methane oxidation rate was observed on pasture. This indicate low emissions of greenhouse gasses from pasture relative to arable land and ley. However, this pattern is not significantly evident and does need more research.

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# Introduction

Soil organic matter (SOM) is the largest terrestrial reservoir of organic carbon (Cheng et al., 2014). Changes in decomposition of SOM affect the atmospheric concentration of CO<sub>2</sub> and small changes in SOM decomposition might have very large effects on global carbon pools and fluxes (Cheng et al., 2014).

The rhizosphere is the layer of soil surrounding and affected by the growing root, and it ranges from a few millimeters up to 1 cm (Philippot et al., 2013). The microbial activity in the rhizosphere soil is usually much higher than in the rest of the soil, partly because the roots exudate labile organic carbon, benefitting microbial activity in a plant-microbe interaction (Philippot et al., 2013). Increased microbial activity affects decomposition of SOM and this has at least two consequences. One, is changes in nutrient availability and nutrient cycling (Dijkstra et al., 2013; Murphy et al., 2015), and another is changes in emission of CO<sub>2</sub> from soil (Cheng et al., 2014). Therefore, changes in the SOM decomposition can have a crucial impact in the ecosystems of soil and on the atmospheric concentrations of CO<sub>2</sub>.

Rhizosphere interactions (activity in the root zone) has a high influence on the fluxes of carbon to and from the soil. One very important part of the rhizosphere interactions is the rhizosphere priming effect (RPE) which is defined as changes in the SOM decomposition rate in response to labile organic carbon input from plant roots (Dijkstra et al., 2013).

The RPE can be divided into positive and negative effects on rhizosphere priming, where a “positive priming effect” is defined as an increase in the decomposition rate of SOM and a “negative priming effect” as a decrease in the decomposition rate (Dijkstra et al., 2013). It is not clear what determines the magnitude and direction of the process. Many factors seem to be involved, and which ones are the dominating factors is still a topic for research (Dijkstra et al., 2013). Some important factors are likely to be nutrient availability, soil properties and vegetation type (Cheng et al., 2003; Dijkstra et al., 2013; Cheng et al., 2014; Murphy et al., 2015). A positive priming effect may be explained by the *microbial mining hypothesis*, suggesting that in soils with low nitrogen availability microbes use the root exudates to release nitrogen by breaking down SOM, thus increasing decomposition rate (Dijkstra et al., 2013; Murphy et al., 2015). A negative priming effect may be explained by the *preferential substrate utilization hypothesis*, suggesting that in soils with high nitrogen availability microbes use labile root

exudates as their carbon source instead of decomposing recalcitrant SOM (Dijkstra et al., 2013; Murphy et al., 2015). This may be the case especially if carbon is limiting microbial activity and not nitrogen (Murphy et al., 2015). Another explanation for negative priming effects is the *competition hypothesis* that propose that microbes and plant compete for the same nutrients (Dijkstra et al., 2013). As plants grow they take up nutrients from soil and this might reduce microbial decomposition (Dijkstra et al., 2013), which may explain negative priming effects on soils with low nutrient availability and therefore high competition for nutrients.

Climate change is likely to affect the RPE through higher CO<sub>2</sub> concentrations in the atmosphere and higher global temperatures, which might sustain a higher vegetational growth rate and are likely to affect the exudation from roots with changes in the RPE as a consequence (Dijkstra et al., 2013; Cheng et al., 2014). One plausible scenario may be that higher rates of root exudates will cause higher rates of decomposition of SOM, resulting in a positive feedback mechanism concerning atmospheric CO<sub>2</sub> and thus accelerating climate change (*microbial mining hypothesis*). On the other hand, it is also a possibility that increasing rates of root exudates into soil will decrease the decomposition rate as more labile organic carbon is available and the microbes will prefer this instead of the more recalcitrant (“old”) organic carbon (*preferential substrate utilization hypothesis*). This will be a negative feedback mechanism slowing down climate change.

It is important to expand the knowledge in this field, as the magnitude and direction of RPE are not very well understood, and contradicting results have been observed. Cheng et al. (2003) found no significant differences in priming due to NPK fertilizing, but did find that plant phenology and temporal variation had significant effects on the RPE. This contradicts the *microbial mining hypothesis*. On the contrary, Dimassi et al. (2014) concluded that priming was mainly controlled by nutrient availability and that tillage had no significant effect on priming, and Zang et al. (2016) observed that increasing N fertilizing caused negative priming effects and decreasing rates of decomposition which resulted in lower emissions of CO<sub>2</sub>. These two later studies thereby support the *microbial mining hypothesis*. Nutrient availability in agricultural soil, and especially nitrogen, is tightly linked to the land use type. Therefore, this study will investigate the RPE on three different land use types.

Methane (CH<sub>4</sub>) is also playing an important role in the carbon cycle of terrestrial ecosystems. Being one of the most important greenhouse gasses (together with CO<sub>2</sub>), and since atmospheric concentrations have increased over the past century, fluxes of methane to and from the atmosphere is an important factor in global climate change (Mancinelli, 1995). Methane is oxidized by certain bacterial enzymes in aerobe soil which represents a flux of methane from the atmosphere into soil (Goulding et al., 1995). However, the process can be interrupted if ammonium (NH<sub>4</sub><sup>+</sup>) in soil occurs in sufficient amounts, as this will cause inhibition by competing with CH<sub>4</sub> for the active site (Goulding et al., 1995). Therefore, as



nitrogen availability have an impact on methane oxidation, this process is also likely to be affected by land use. Addition of labile organic carbon to the soil does not affect these specialized bacteria directly, but it might increase the demand for nitrogen for the bacteria and thus lower the concentration of  $\text{NH}_4^+$ , which in turn might result in higher rates of methane oxidation. It should be mentioned that even if methane oxidizing bacteria produce  $\text{CO}_2$ , being another greenhouse gas, their total effect is a greenhouse gas reduction, as most of the consumed methane is transferred into organic carbon as biomass (Mancinelli, 1995).

This study aimed to test whether differences in rhizosphere priming and methane oxidation, respectively, could be observed between three different types of agricultural land uses; arable land, pasture and ley. Ley in this study refers to grassland which is harvested mechanically, in opposition to pasture which is only grazed by animals. Arable land in this study is defined as done by Eurostat (2015):

“land worked (ploughed or tilled) regularly, generally under a system of crop rotation”.

Arable land of this study is fertilized with manure regularly while the pasture and ley is not fertilized. While the ley in this study has not been fertilized for at least ten years (possibly much longer) it contains different species of legumes that fix nitrogen from the atmosphere. Therefore, ley was not expected to be as low in nitrogen availability as the pasture. The crop on the arable land was rye (*Secale cereale*) having a height above soil of 10-15 cm at the sampling time.

It was hypothesized that the largest (positive) priming effect would occur in fields with low nitrogen availability, as predicted in the *microbial mining hypothesis*; pasture was expected to show higher rates of priming compared to ley and especially arable land. Methane oxidation was expected to be highest in soils with low nitrogen availability as the methanotrophs switch to consuming  $\text{NH}_4^+$  if this occur in high concentrations of instead of consuming  $\text{CH}_4$ . As arable land is fertilized and ley contains nitrogen-fixing plant species, methane oxidation might be inhibited in these types of land use. Therefore, methane oxidation was hypothesized to be highest in pasture soil.

The results can be used to broaden the discussion of land use impact on global climate in terms of RPE and soil ecology processes. Soil properties (nitrogen availability, pH, SOM % and soil water content) were also measured and it was tested whether any significant differences occurred between land uses, and whether it correlated with priming or methane oxidation.



# Material and Methods

## Experimental design and site description

The priming effect were estimated by adding labile organic carbon ( $^{13}\text{C}$ -glucose) to one soil sample and comparing it to a control (an identical soil sample without additional glucose). By isotope-marking the added carbon it was possible to distinguish between the  $\text{CO}_2$  emissions derived from SOM decomposition (including the priming effect) and the  $\text{CO}_2$  from microbial respiration of glucose, the latter being marked with  $^{13}\text{C}$ . An isotope analyzer (*Picarro G2201-i Analyzer*) was used to measure the “potential priming” of the soil samples, meaning that a relatively high amount of glucose was added to the soil to get all potential priming within two days.

Methane oxidation was measured by injecting  $\text{CH}_4$  to the soil samples and then measuring the changes in  $\text{CH}_4$  concentration with the *Picarro analyzer* in the same way as described above. This experiment was carried out in the Ecology building at Lund University between the 19<sup>th</sup> of April and the 23<sup>rd</sup> of April.

Differences in priming were investigated between arable land, pasture and ley. All three experimental fields were situated close to each other near a small farm just outside the village of Vomb in southern Sweden, and have been managed continuously in the same way for at least ten years (probably much longer). From each land use type, four replicates were taken with five meters between in a line parallel with a small unpaved road. This, to avoid differences in nitrogen concentrations between replicates due to emissions of nitrogen oxides from cars. Each replicate consisted of three cores of soil from 0 to 15 cm below surface.

## Limitations of this study

Due to the limited time for experimental work and data analysis, potential differences between organic and conventionally managed fields are not a part of this study. This is for other studies to be done. Furthermore, variation between different fields of the same land use type is not tested either. Only the variation inside one field of the same land use type is tested.

## Measurements of CO<sub>2</sub> and CH<sub>4</sub>

CO<sub>2</sub> and CH<sub>4</sub> concentrations and atom%<sup>13</sup>C was measured using the *Picarro Analyzer*. The workflow was as following. Soil samples were taken out from the fridge 1 hour before measurements. Control samples received additional water to adjust water content to 40 % of water holding capacity. 8 minutes before closure of chambers the samples that did not had any water added, received 6 atom% <sup>13</sup>C-glucose in concentrations that were adjusted so that these samples would also reach a water content of 40 % of water holding capacity. The concentration ratios after addition to soil were 1,0 mg glucose per g soil dry weight (dw).

A sequence was created to program the *Picarro analyzer* when to measure each sample and for how long:

- 1 minute of flushing the *Picarro analyzer* with fresh air
- 5 minutes of carbon trapping
- 3 minutes of measuring

(Continuing to the next sample...)

After one round finished a new began automatically. Samples were put into chambers and flushed with air and the lids were closed 15 min before the measurement for each sample began. 0,25 ml air with methane was injected immediately after lids were closed, resulting in a methane concentration of approximately 140 ppm in the headspace of the chambers. The *Picarro analyzer* was run for 48 hours in total, performing measurements every 3<sup>rd</sup> second. After 24 hours, lids were opened to release vacuum and the chambers were flushed for 1 minute. Methane was re-injected as above. Lids were opened after 48 hours and experiment ended.

In total 24 samples were analyzed, as 3 different fields were chosen, 4 replicates analyzed for each field and an equal number of samples analyzed as controls. As the *Picarro analyzer* could only measure a maximum of 14 samples at a time, the experiment was divided into two groups of samples, two replicates with controls in the first group and the rest in the second group. The first group was measured from the 19<sup>th</sup> to the 21<sup>st</sup> of April and the other from the 21<sup>st</sup> to the 23<sup>rd</sup> of April. This gave a small difference in time between measurements of the two groups of replicates. However, as samples had an initial incubation time of 6 days before the experiment, and were kept in refrigerator to minimize microbial activity, no significant differences between replicates were expected due to this, and couldn't be observed in the results either.

## Calculations and statistics

The priming effect was calculated by partitioning the CO<sub>2</sub> flux from the soil into glucose- and soil-derived CO<sub>2</sub> using equation (1). The calculated P<sub>gluc</sub> is the proportion of CO<sub>2</sub> deriving from glucose, and thus the rest is the CO<sub>2</sub> derived from decomposition of SOM. The priming effect was calculated as the difference in CO<sub>2</sub> fluxes between sample with glucose and control when taking away the glucose-derived CO<sub>2</sub>.

$$P_{gluc} = (atom\%^{13}C_{sample} - atom\%^{13}C_0) / (atom\%^{13}C_{gluc} - atom\%^{13}C_0) \quad (1)$$

Where:

P<sub>gluc</sub> = proportion of total CO<sub>2</sub> derived from glucose

Atom%<sup>13</sup>C<sub>sample</sub> = Atom%<sup>13</sup>C-CO<sub>2</sub> signature for the sample at each measuring point

Atom%<sup>13</sup>C<sub>0</sub> = Atom%<sup>13</sup>C-CO<sub>2</sub> signature for the control treatment

Atom%<sup>13</sup>C<sub>gluc</sub> = Atom%<sup>13</sup>C of the added glucose

Tests of significance on the results were performed by the software *Statistica*. A two-way ANOVA was performed when testing 2 variables at the same time (land use and glucose-addition). This was the case when testing respiration and methane oxidation for significant differences with respect to both land use and glucose addition. A one-way ANOVA was performed when testing only one variable (land use). This was the case when testing differences in soil nitrogen levels between land uses, and when testing differences between priming and land use. All data were initially tested without being log<sub>10</sub>-transformed, but to ensure a normal distribution and a homogeneity of variances, two important assumptions of the test, most data was also log<sub>10</sub>-transformed before a redoing of the statistical tests. In the results, only the test of methane oxidation was performed with the original data, while all other tests were done using the log<sub>10</sub>-transformed data.

Linear regression was performed in *Microsoft Excel 2016* to calculate R<sup>2</sup> values for both priming as a dependent variable on total inorganic nitrogen, and for a correlation between methane oxidation and rates of respiration.



# Results and analysis

## Soil properties

Total inorganic nitrogen was significantly lower for pasture compared to arable land ( $p < 0.001$ ) and ley ( $p < 0.001$ ), but no significant differences were observed between arable land and ley ( $p = 0.249$ ). The concentration of  $\text{NH}_4^+$  was significantly higher in ley soil compared to the other two land uses ( $p < 0.001$ ). A complete list of nitrogen content ( $\text{NO}_3^-$ ,  $\text{NH}_4^+$  and total) for all replicates can be found in Table 1 in Appendix A. Significant differences were found for pH ( $P < 0.001$ ), but no significant differences in SOM % were found ( $P = 0.080$ ).

**Table 1** Soil properties (average of all replicates) for arable land, pasture and ley, obtained from soil samples used in the study.

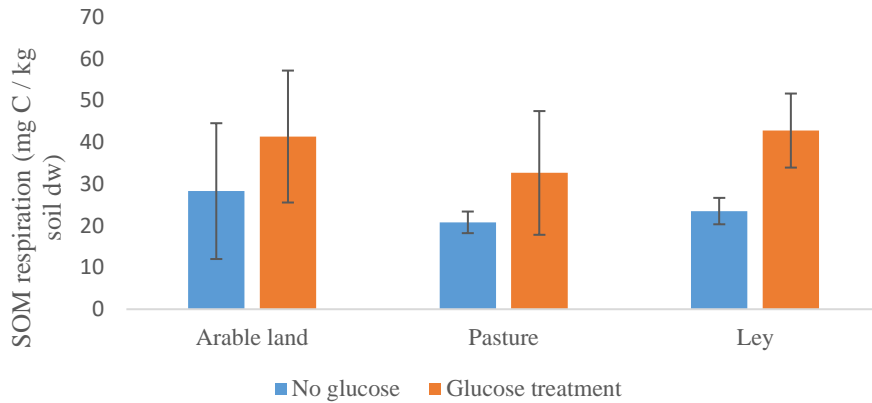
Land use	SOM %	pH	$\text{NO}_3^-$ (ug N/g soil)	$\text{NH}_4^+$ (ug N/g soil)	total inorganic nitrogen (ug N/g soil)
Arable land	3.76	7.33	4.17	1.63	5.80
Pasture	3.20	6.43	0.31	1.19	1.50
Ley	3.38	6.06	4.14	4.84	8.98

## SOM respiration and the rhizosphere priming effect

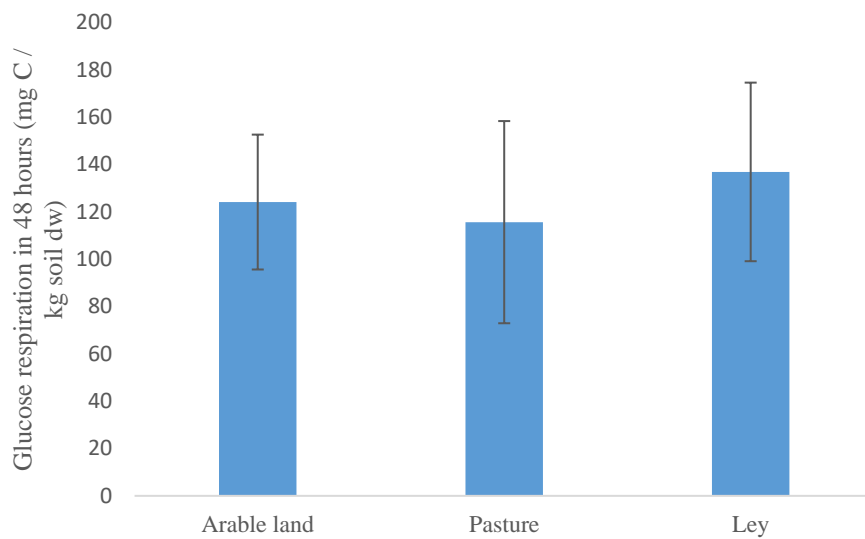
Positive rates of priming were observed in all land uses ( $p < 0.01$ ), as significant differences were observed in SOM respiration between glucose treatments and the controls (figure 1 and figure 4).

SOM respiration in 48 hours for the three land use types varied between 21-28 mg C per kg soil dw in controls, and between 33-43 mg C per kg soil dw in glucose amended samples (figure 1). The lowest SOM respiration was observed in pasture while higher rates were observed in arable land and ley (figure 1). The same pattern was observed for glucose respiration (figure 2) and total respiration (figure 3). However, the differences between land uses were not significant for either SOM

respiration ( $p=0.368$ ), glucose respiration ( $p=0.683$ ) or total respiration ( $p=0.538$ ), respectively.

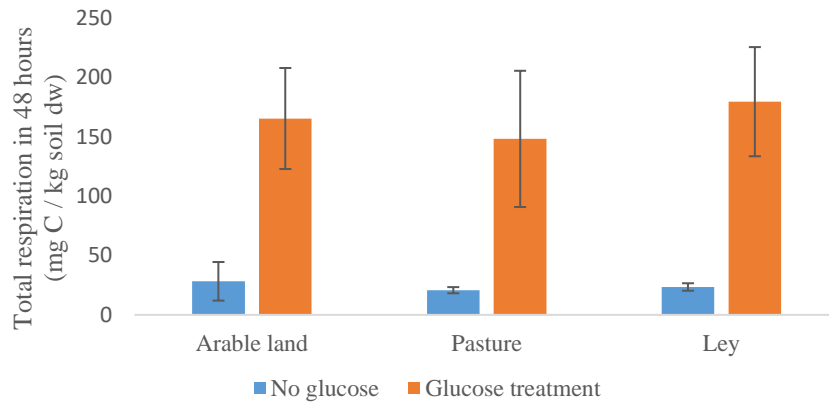


**Figure 1** Cumulative SOM respiration in 48 hours for three land use types. Significant differences were observed between glucose treatments and controls for all land uses ( $p<0.01$ ), but no significant differences were observed between land uses ( $p=0.368$ ). Error bars represent standard deviation of the mean.



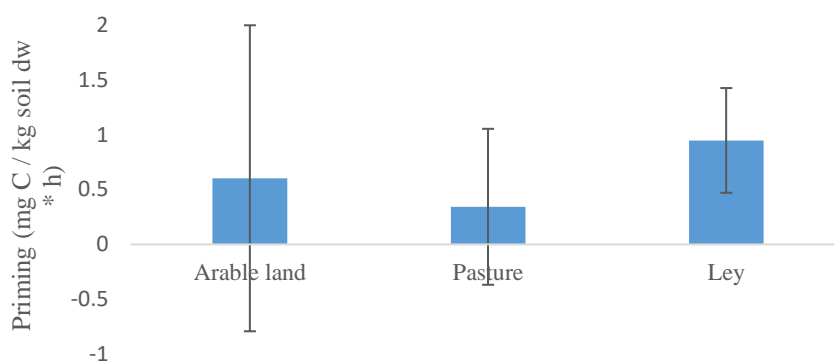
**Figure 2** Cumulative glucose respiration in 48 hours for three land uses types. No significant differences due to land use were observed ( $p=0.683$ ). Error bars represent standard deviation of the mean.





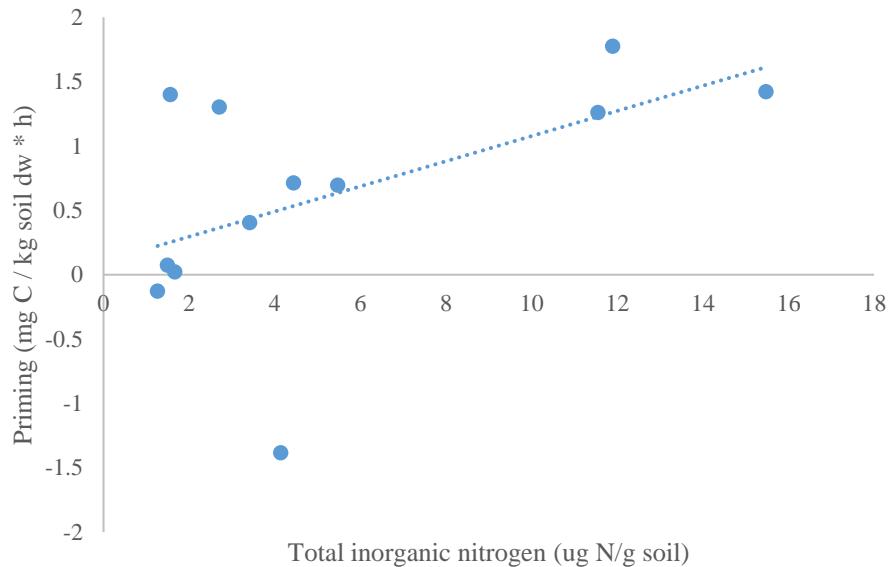
**Figure 3** Cumulative total respiration (SOM and glucose) in 48 hours for three land uses types. No significant differences due to land use were observed ( $p=0.538$ ). Error bars represent standard deviation of the mean.

Considering the magnitude in SOM respiration for the respective controls (figure 1), SOM respiration increased relatively more for ley (82% increase) compared to arable land (46% increase), when treated with glucose. This explains that the priming effect was calculated higher in ley compared to arable land (figure 4). Despite a higher relative increase in SOM respiration for pasture (57%) compared to arable land, the lowest priming effect in absolute numbers was observed in pasture, with the highest being in ley and an intermediate priming effect in arable land (figure 4). In pasture a priming effect of 0.34 mg C per kg soil dw and hour was observed, compared to 0.6 and 0.9 for arable land and ley, respectively. However, the differences in priming between land uses were not significant ( $p=0.674$ ).



**Figure 4** The priming effect per hour and land use type. No significant differences due to land uses were observed ( $p=0.674$ ). Error bars represent standard deviation of the mean.

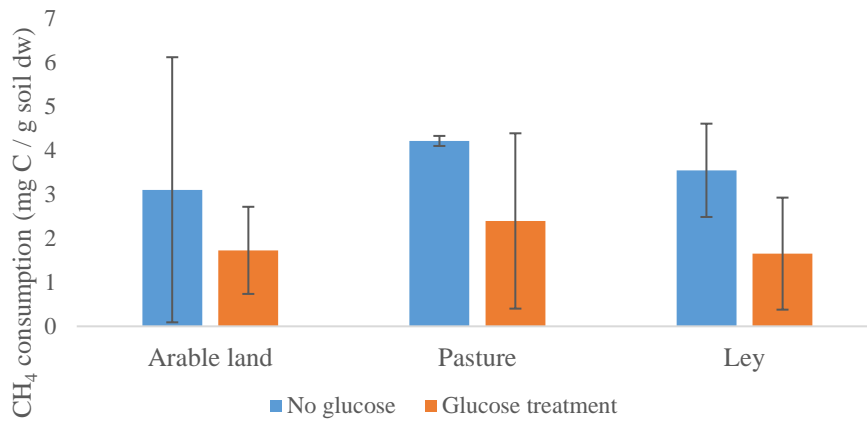
Priming was positively correlated with nitrogen availability (figure 5) meaning that a higher nitrogen availability correlated with a higher priming effect. The correlation would have been even stronger ( $R^2 = 0.45$ ) without one outlier showing a relatively large negative priming effect.



**Figure 5** A scatterplot shows priming effects as a dependent variable of nitrogen availability (4 replicates from each of the three land use types). Priming effect on the y-axis and total inorganic nitrogen on the x-axis ( $\text{NO}_3^-$  and  $\text{NH}_4^+$ ). A linear regression revealed an  $R^2$  value of 0.28.

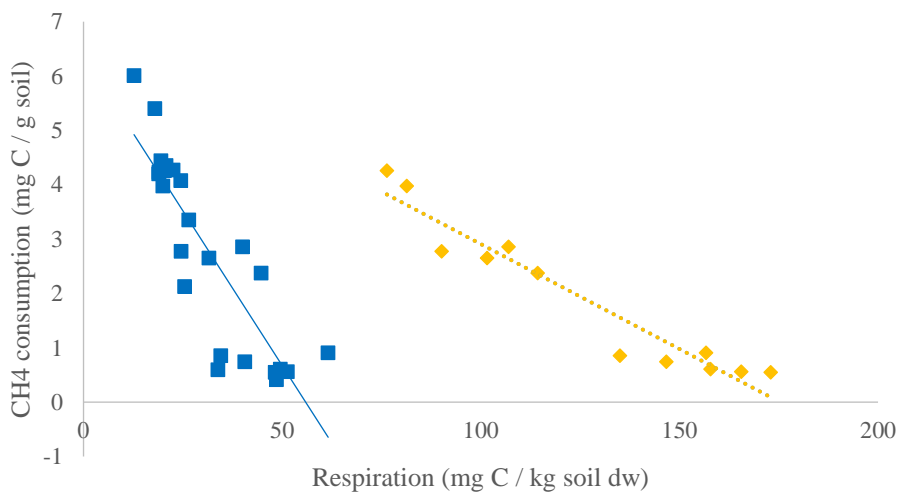
## Methane oxidation

Rates of methane oxidation among the controls ranged between 3.1-4.2 mg C per g soil dw, the highest rates being observed in pasture and the lowest in arable land, with an intermediate level in ley (figure 6). In the glucose treatments, methane oxidation ranged between 1.7-2.4 mg C per g soil dw, with pasture showing the highest rates, and arable land and ley having similarly lower rates figure 6). However, as for the SOM decomposition (figure 1) and priming (figure 4) no significant difference in methane oxidation was observed between land uses ( $p=0.512$ ).



**Figure 6** Cumulative methane oxidation in 48 hours for three land uses types. No significant differences due to land use were observed ( $p=0.512$ ). Error bars represent standard deviation of the mean.

A strong negative relationship was observed between methane oxidation and rates of respiration (figure 7), as methane oxidation decreased with higher rates of both SOM respiration ( $R^2 = 0.75$ ) and glucose respiration ( $R^2 = 0.93$ ).



**Figure 7** A scatter plot shows a negative correlation between  $CH_4$  oxidation on the y-axis and both SOM respiration and glucose respiration on the x-axis. A linear regression revealed  $R^2$  values of 0.75 and 0.93 for SOM respiration and Glucose respiration respectively.

Although not significant, a pattern can be observed as pasture on average had the lowest SOM respiration, and the highest CH<sub>4</sub> oxidation rate, which both result in low emissions of carbon relative to arable land and ley. This was also illustrated by the priming effect which was lowest in pasture (figure 4). This is going to be evaluated in the discussion.

## Discussion

According to one hypothesis of this study, the priming effect was expected to be largest in fields with low nitrogen availability, as predicted in the *microbial mining hypothesis* (Dijkstra et al., 2013; Murphy et al., 2015). Non-fertilized pastures were expected to have lower N concentrations and therefore to show higher rates of priming compared to ley and arable land. This was not the case, even if pasture had the lowest N concentrations. On the contrary, although not significantly, the lowest rates of SOM respiration and the lowest priming effect was observed in pasture (0.34 mg C per kg soil dw) compared to arable land and ley (0.6 and 0.9 mg C per kg soil dw, respectively). This does not correspond with the *microbial mining hypothesis* (Dijkstra et al., 2013; Murphy et al., 2015).

The results showed on average positive priming effects meaning that neither the *competition hypothesis* nor the *preferential substrate utilization hypothesis* can be used as explanations. This calls for more theories on the subject.

A fourth hypothesis can be used to explain the results, the *stoichiometric decomposition hypothesis*. According to this, increased rates of SOM decomposition will occur when both nitrogen and carbon input matches stoichiometric C and N ratios demanded by the microbes (Chen et al., 2014). In other words, sufficiently high availability of both nitrogen and carbon is needed to reach a high, positive priming effect. In this study a positive correlation between nitrogen availability and priming was observed (figure 3) which supports this theory, and indicate that, even if the relationship was not clear for land uses, the nitrogen availability itself is likely to have an important impact on priming. Chen et al. (2014) concluded that input of labile organic carbon acts as a primer for SOM decomposition by switching microbes to an active state and producing extracellular enzymes for breakdown of SOM, but high availability of inorganic nitrogen allows the microbes to produce more extracellular enzymes, thereby controlling the process of priming. Thus, the mechanism of *stoichiometric decomposition* is likely to be responsible for the priming effect when availability of both C and N are high, while the mechanism of *microbial mining* might be responsible for the priming effect during input of labile organic carbon when availability of N is low. These mechanisms might even coexist in the same soil and have influence on the priming effect at the same time due to different microbial groups (Chen et al., 2014). This view is also shared by Cheng and Kuzyakov (2005) arguing that different

mechanisms operate in combination due to spatial and temporal variations in the availability of C and N.

Understanding of the mechanisms behind the priming effect is needed to be able to develop agricultural practices that emit the lowest possible quantities of CO<sub>2</sub> or even act as atmospheric C sink. Whether a soil ecosystem acts as C sink or source is decided by the net ecosystem production (NEP), which is the difference between gross primary production and ecosystem respiration (Ehtesham and Bengtson, 2017). Increasing global temperature and atmospheric concentration of CO<sub>2</sub>, in combination with sufficient availability of nitrogen, might not only lead to higher primary production but also increased exudation of labile organic carbon from roots and increasing decomposition of SOM (Bengtson et al., 2012; Cheng et al., 2014). This scenario would lead to a positive feedback loop, with increasing concentration of CO<sub>2</sub> in the atmosphere and devastating consequences for global climate. In opposition, Ehtesham and Bengtson (2017) found that labile C input reduced SOM respiration. Furthermore, as increasing nutrient availability is likely to occur with increasing SOM decomposition (Dijkstra et al., 2013; Murphy et al., 2015), this could also lead to increased plant growth, with an increased uptake of CO<sub>2</sub> by plants as a consequence, representing a negative feedback loop. The contradiction of the studies mentioned and the lack of significant results in this study itself, emphasizes the importance of more research in this field and a need for stronger evidence.

Methane oxidation was expected to be highest in soils with low nitrogen availability, and in terms of land use this was expected to be pasture soil. No significant differences were observed between land uses in this study, but the results give some indication of a possible trend. The highest methane oxidation rate was observed in pasture, which corresponds with the hypothesis that the highest methane oxidation occurs in soil with low nitrogen availability, especially if NH<sub>4</sub><sup>+</sup> is low (Goulding et al., 1995). Both total inorganic nitrogen and ammonium was significantly lower in pasture compared to other land uses. As CH<sub>4</sub> is an important greenhouse gas, and since its atmospheric concentrations have increased over the past century, it is playing an important role in global climate change (Mancinelli, 1995). To understand how to optimize methane oxidation in soil, via methanotrophs that are keeping down the levels of methane in the atmosphere, will be important in mitigating global climate change. Agricultural land use may be a key factor to this (Goulding et al., 1995), even though significant differences was not observed in this study between the three land uses tested. According to Mancinelli (1995) application of ammonia fertilizers on agricultural land has resulted in a significantly reduced capacity for methane oxidation.

Methane oxidation was negatively correlated with rates of respiration in soil as higher respiration (both SOM and glucose) correlated with decreasing methane oxidation. This was unexpected as the addition of glucose results in an increased microbial activity and thereby an uptake of nitrogen, meaning less available nitrogen in soil. This would give incitement to the methanotrophs to oxidize

methane instead of  $\text{NH}_4^+$  (Goulding et al., 1995). One explanation might be that the time frame of two days was not enough to activate these mechanisms. Another explanation could be that concentrations of  $\text{NH}_4^+$  were too low in the tested soils. However, this is not likely as Goulding et al. (1995) observed that a relatively low annual application of 22 kg N per hectare and year, during 15 years, was enough to reduce  $\text{CH}_4$  by 41 % compared to unfertilized pasture. Also, the fact that observed levels of methane oxidation were not only unaffected of respiration but showed a negative correlation, makes other explanations needed. The decrease in methane oxidation might be due to oxygen deficit in soil provoked by the intense increase in microbial respiration and activity, as oxygen availability is a controlling factor of methane oxidation (Mancinelli, 1995). High microbial activity might also lead to competition between other nutrients (e.g. fixed nitrate and copper) and thus inhibit methane oxidation (Mancinelli, 1995).

As described above, the results of this study indicate that land use alone did not have significant effects on the RPE and thus no effects via priming on the atmospheric concentrations of  $\text{CO}_2$ . Whether this corresponds with reality or is due to the design of the experiment is not clear. The number of replicates were adjusted from 5 to 4 due to technical limitations and the time frame of the study. However, as within-site variation was larger than variation between sites, more replicates seem necessary to make safe conclusions in this field.

Nevertheless, the results give some interesting indications that should be investigated in more detail in further studies. Pasture on average has the lowest SOM respiration, and the highest methane oxidation rate, both resulting in lower emissions of carbon relative to arable land and ley. This is also illustrated by the priming effect which is lowest in pasture. If this tendency could be stated with significance, environmental recommendations could be given in terms of limiting carbon emissions due to agricultural land use. As pasture and soil with low nitrogen availability may have a higher ability of sequestration of carbon, and thus a smaller impact on global climate compared to arable land and ley, one recommendation could be that grazing animals would be less harmful for global climate than growing crops as feed for domestic animals. This view is not supported by significant results and does need more research. Of course, other aspects also need to be considered, for example emissions of  $\text{CH}_4$  from domestic ruminants, and whether type of feed etc. affects emissions from these.

As SOM content was slightly higher in arable land (3.76%) than in pasture (3.20%) it might seem conflicting to draw the conclusion that carbon sequestration is higher in pasture, but the high content of SOM in arable land may be explained by the regularly application of manure which cause increased content of SOM. From this perspective, a relatively high SOM content seems to be sustained in pasture. In comparison, ley had an intermediate level of 3.38 %.

As the difference between replicates showed, a high variation exists in each land use type and thus a high spatial variation inside each land use type in general

is likely, both in terms of soil properties but also in terms of soil processes like priming and methane oxidation. To draw conclusions that can be used in decision making, more than one field of each land use type will be necessary, as the sites used for this study might not be representative for these land uses in general. Especially ley can be very different from field to field in terms of nutrients and management. Ley is a broad term sometimes including fertilized fields, and differences in cutting regime occur. Some leys can even be grazed after harvesting and thus the distinguishing between ley and pasture becomes less clear. Two replicates of the ley in this study had an availability of nitrogen close to those of pasture while two had as high values as the highest in arable land (Table 1 in Appendix A). Whether those nitrogen levels derive from much earlier fertilization or because of legumes in the ley is not clear. But as legumes occur in the ley, it is not a surprise that the nitrogen availability is higher than in the pasture. The legumes might also explain the extremely high variation in nitrogen as the legumes appear in clusters in the field.

In this study, there are uncertainties in the soil sampling and the methods of measuring might also be a source of error, which could be minimized with more replicates, as mentioned above. Long term experiments might also give more liable results than to extract potential priming in two days like this experiment aimed to do. Another factor to be aware of is that the measurements were done in a lab, which means that soil samples were incubated and isolated from surrounding ecology, and that “real” plants are not present when doing the measurements (Cheng et al., 2003).



## Conclusion

The priming effect was observed for all land uses with significant increases in SOM decomposition in soil treated with glucose. However, priming or SOM decomposition did not differ significantly between land uses (arable land, pasture and ley). Also, no significant differences were found in methane oxidation between land uses. However, a pattern can be observed, as pasture on average has the lowest SOM respiration, and the highest methane oxidation rate, which both results in low emissions of carbon relative to arable land and ley. From a climate change perspective, this indicates that pasture might be the more climate friendly land use type, if only focusing on the emissions of greenhouse gasses from the soil. These relationships should be investigated in more detail in future studies. One suggestion is to expand the number of replicates in a similar study, and to perform a study of the priming effect on a longer time scale, e.g. 2 weeks or even more.



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# Appendix A

**Table 1** Nitrogen content for all replicates. Observe the large variation for arable land and ley.

Sample	Soil Type	Replicate	NO <sub>3</sub> -N (ug N/g soil)	NH <sub>4</sub> -N (ug N/gsoil)	total inorganic nitrogen
1	Arable land	1	1.37	1.34	2.71
2	Arable land	2	2.86	1.27	4.14
3	Arable land	3	9.10	2.80	11.90
4	Arable land	4	3.33	1.10	4.44
6	Pasture	1	0.33	1.24	1.56
7	Pasture	2	0.29	0.97	1.26
8	Pasture	3	0.29	1.20	1.49
9	Pasture	4	0.33	1.34	1.67
11	Ley	1	6.93	8.54	15.48
12	Ley	2	1.54	1.88	3.42
13	Ley	3	2.71	2.76	5.47
14	Ley	4	5.38	6.17	11.55



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