The vertical distribution of microbial biomass in the topsoil of four differently-aged heathland communities
Abstract
This research investigates the effect of four differently-aged heathland communities on the vertical distribution of microbial biomass in the topsoil. The research is conducted in Oldebroek, the Netherlands in April and May 2015. The ages of the heathland communities are 2, 15, 22 and 31, and are called Pioneer, Young, Middle and Old community respectively. At each community, the soil is divided into three soil layers (0-2, 2-5 and 5-10 cm), from which six samples are taken at all heathland communities. For measuring microbial biomass concentration, fumigated-extraction (FE) and substrate-induced respiration (SIR) methods are used. The results of both methods are expressed per gram dry weight and per gram organic material (OM), as this gives an indication of the amount of microbial biomass in the soil and of the soil quality. The FE method indicates significant differences between the Pioneer and Middle community and Young and Middle community for the 0-2 cm soil layer. Additionally a significant difference between the Young and Middle community and Young and Old community for the 2-5 cm soil layer and between the Young and Old community for the 5-10 cm soil layer when based per gram dry weight. Furthermore, it shows significant differences between the Pioneer and Old community and Young and Old community for the 5-10 cm soil layer when based per gram OM. The SIR method shows no significant differences for both the per gram dry weight or gram OM calculations. This might be explained by an underestimation of the SIR method. However, to examine this discrepancy it is recommended to perform a third method for measuring the microbial biomass, such as the ATP extraction method.
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**Introduction**

**Background information**

The driving force behind mineralization of soil organic matter and plant and animal litter are the microorganisms present in the soil. These microorganisms are by definition smaller than $5 \times 10^3 \mu m^3$ (Gonzalez-Quinones et al., 2011). The total amount of microorganisms in the soil is referred to as microbial biomass and this makes up about 1-3% of the soil organic carbon. Even though the microbial biomass contributes such a small percentage to the total soil organic carbon, these microorganisms are responsible for the release of carbon dioxide ($CO_2$) and mineralization of nutrients in inorganic form back into the ecosystem. These nutrients are then available again for plant uptake (Martens, 1995). Besides mineralization, microbial biomass is also responsible for other processes in the soil, such as nitrogen fixation, denitrification and nitrification and transformation of metals. All these processes are important for sustaining ecosystems (Panikov, 1999).

The soil organic matter required by microorganisms for energy can only be taken up in a dissolved form. In a dissolved form, the organic matter can be transported to the microorganisms and transferred through the microbial membranes (Marschner & Kalbitz, 2003). Therefore, factors that affect the amount of dissolved organic matter also affect the rate of mineralization. These factors are for example the pH, water content and temperature of the soil. Anderson and Domsch (1993) discovered that the ratio of microbial carbon to total soil carbon increased with increasing pH and decreased with decreasing pH compared to the microbial biomass conditions at a neutral pH. When pH of the soil is high, desorption of organic matter occurs, which can increase the mineralization (Curtin et al., 1998). However, the specific respiration rate of the microbial biomass increased under more acid conditions. This could be due to microorganisms in acid soils require more energy for cell maintenance and less energy is invested in growth (Anderson & Domsch, 1993). Furthermore, both an increase in water content and temperature to a certain degree, increases the rate of mineralization (Curtin et al., 1998).

The above factors all influence the activity of microbial biomass present in a soil. However, microbial biomass can only increase when the total soil organic matter is not a limiting factor (Curtin et al., 2012). The highest quantity of microbial biomass is therefore mostly found in the soil layer with the highest content of soil organic matter. Murphy et al. (1998) investigated the microbial biomass distribution for two types of soil with soil depth. They reported that for both soils 55% of the microbial biomass was concentrated in the surface layer (0-10 cm). Below the surface layer, the microbial biomass declined rapidly with depth. Doran (1987) showed that the highest concentration of microbial biomass can be located below the surface layer of ploughed soils, where transport of soil organic matter vertically into the soil occurs. Additionally, Woods (1989) reported similar results for an undisturbed and a disturbed soil. The amount of microbial biomass in the surface layer of the undisturbed soil was significantly higher than the disturbed soil.

The rate of mineralization is also determined by the quality of soil organic matter. The quality of soil is expressed as the ratio of microbial biomass over organic matter. A relative low ratio might indicate the dominance of old, less degradable organic matter whereas a relative high ratio might indicate the dominance of fresh, easily degradable organic matter (Eiland et al., 2001). An increase in less degradable material, such as lignin, slows down the mineralization rate. This is due to that lignified material is resistant to microbial mineralization, and can only be decomposed by certain species,
particularly fungi (Austin & Ballaré, 2010). The quality of soil organic matter influences the microbial efficiency, which is an indication of the amount of organic matter converted into microbial biomass and CO₂. The microbial efficiency declines when the quality of the organic matter decreases (Frey et al., 2013). This indicates that more organic matter is converted into CO₂ instead of microbial biomass.

**Research question and hypothesis**

In this report, the research question whether four differently-aged heathland communities have an effect on the vertical distribution of microbial biomass in the topsoil will be discussed. The fieldwork was conducted in a heathland near Oldebroek, the Netherlands. The most dominant heathland species at the location is *Calluna Vulgaris* (L.) Hull. For the research, four differently-aged heathland communities were used. The ages of the communities are 2, 15, 22 and 31 (Kopittke et al., 2012). According to Barclay-Estrup and Gimmingham (1969), the phases of the communities can be classified as pioneer, building, mature and degenerate respectively when the life cycle of Calluna is considered. In this report, they will be referred to as Pioneer, Young, Middle and Old community. One of the characteristics of Calluna is that the lignified material increases with increasing age. Furthermore, the amount of aboveground biomass is highest during the mature phase, and is reduced in the degenerate phase (Gimingham, 1985). Based on this knowledge, it is hypothesised that the Pioneer and Young community will have the highest amount of microbial biomass and highest soil quality for all soil layers compared to the Middle and Old community. This is based on the expected amount on lignin present in the organic matter.

To measure the amount of microbial biomass and quality of the soil two techniques were used, namely the fumigated-extraction (FE) and substrate-induced respiration (SIR) method. The results of both methods will be given on a dry-weight basis and organic matter (OM) basis. The results based on dry-weight will give an indication of the amount of microbial biomass per soil layer per heathland community, whereas the results based on OM will give an indication of the relative soil quality per soil layer per heathland community.

**Relevance**

Previous research has already been conducted at the research location. The findings of this research might therefore be a relevant contribution to the already existing knowledge about the location. Furthermore, the concentration of microbial biomass might be an indication of the amount of CO₂ released into the atmosphere. As stated before, low quality soil emit a higher amount of CO₂, due to a decrease in microbial efficiency. This research might indicate which soil layer of each heathland community has a higher quality, by comparing the ratio of microbial biomass over OM between the heathland communities per soil layer. This knowledge might influence the management practises of heathland, in order to minimize the CO₂ input into the atmosphere from heathland soils.
Methods

Research site

The research was conducted in a heathland in Oldebroek, the Netherlands, at the coordinates 52°24’N 5°55E. This location has been the research site for many investigations related to heathland (van Meeteren et al., 2008; Kopittke et al., 2012; Kopittke et al., 2013). The dominant heathland species is Calluna. During the fieldwork in April and May 2015, four communities of differently-aged heathland within a 50m x 50 m plot were identified: the Pioneer, Young, Middle and Old communities. The age difference of the communities was considered to act as the main differing characteristic for the experiment when statistical tests were conducted.

The soil type at the research site is identified as an acid sandy Haplic Podzol, with a mormoder humus form by van Meeteren et al. (2008). Furthermore, it is a well-drained heathland with a low concentration of nutrients. The pH of the topsoil (0-10 cm) is approximately 3.9.

Sampling method

At the four heathland communities, 6 samples of the soil layers 0-2, 2-5 and 5-10 cm were collected. To obtain the samples, a soil core of the top 10 cm was exhumed and divided into the three samples, resulting in a total amount of 72 samples (n=72). No distinction was made based on the horizons of the soil at each location. However, this does not impede the research as the research question is concerned with the vertical distribution of microbial biomass of the topsoil of each community. The soil cores were retrieved using a tube with a diameter of 4.3 cm, after which the samples were stored in a refrigerator at 7°Celsius. To ensure obtaining sufficient material, 8 repetitions (0.0116 m²) were required with the tube for the 2-5 and 5-10 cm soil samples per sample. For the 0-2 cm soil samples 20 repetitions (0.029 m²) per sample were collected. These repetitions were conducted in an area of 1 m² per sample. The sampling locations and the method of sampling are illustrated in figure 1 and 2.

![Figure 1: Soil sample locations of each heathland community. Based on figure of Kopittke et al., 2013.](image1)

![Figure 2: Overview of the sampling method based on one sample site](image2)
Due to the relative small size of the research area, it is assumed that the pH and temperature of the soil samples per layer are not significantly different. Therefore it is expected that these soil characteristics have no significant influence on the spatial distribution of microbial biomass.

**Laboratory activities**

As explained in the introduction, to measure the amount of microbial biomass and quality of the soil two techniques were used, namely the fumigated-extraction (FE) and substrate-induced respiration (SIR) method. The results of both methods will be given on a dry-weight basis and on an OM basis.

To obtain the dry-weight, 3 grams of each sample was dried in an oven for 48 hours. The samples of 0-2 and 2-5 cm depth were dried at 70°C and the samples of 5-10 cm at 105°C. Furthermore, the OM content of each sample was obtained by using the loss on ignition (LOI) method (Navarro et al., 1993). Each sample was placed in an oven at 500°C for 16 hours. Both the dry-weight and loss on ignition was measured in duplicate. The mean values for dry weight, moisture content, gram LOI and percentage LOI for all samples can be found in appendix A.

For both methods, the samples were examined to exclude stones, roots and other plant litter. When the samples were not used, they were kept refrigerated, in order to prevent the microbial biomass from reproducing. This would potentially impede the research. The FE method used in the report is based on the method by Vance et al. (1987). For the FE method each sample was subdivided into two groups: the first group for fumigation with amylene-stabilized chloroform and extraction with 0.05 M \( \text{K}_2\text{SO}_4 \) and the second group for direct extraction with 0.05 M \( \text{K}_2\text{SO}_4 \). For both groups, 3 grams of the organic soil layer (0-2 cm) and 10 grams of the mineral soil layers (2-5 and 5-10 cm) were weighed in 100 ml jars. The chloroform fumigation was done in a fume hood in the laboratories of the Faculty of Science, University of Amsterdam, and lasted 24 hours. Before the samples were removed from the fume hood, they were aerated in order to remove all chloroform. After that, 40 ml of 0.05 M \( \text{K}_2\text{SO}_4 \) was added to each jar, after which they were shaken for one hour. Then the extracts were filtrated, under vacuum conditions, using filter papers. These last steps were also conducted on the direct extraction samples. Subsequently, the total organic carbon (TOC) content of both groups were obtained using a total organic carbon analyzer (Elementar Vario TOC cube). The microbial biomass (in mg C g\(^{-1}\) soil) was calculated by the difference in TOC content between the fumigated and the non-fumigated samples divided by an extraction factor of 0.45.

The SIR method is based on the method used by Sawada et al. (2009). For the SIR method, the samples were given 24 hours to acclimatize. Furthermore, water content was equalized for all samples per soil layer per heathland community. This is done in order to prevent an uneven influence of the water content on the activity of the microbial biomass. To conduct the experiment, 9 g of the organic layer samples (0-2 cm) and 30 grams of the mineral layer samples (2-5 and 5-10) were put in 100 ml jars. These jars were placed in the respicord at a constant temperature of 20°C. The respicord measured the rate of respiration (in \( \mu \text{g C/h} \)) each half hour for all samples. After approximately 15 hours, 50 mg of liquid glucose was added to 0-2 cm and 2-5 cm soil layer and 25 mg of liquid glucose was added to the 5-10 cm soil layer, which is assumed to be an excessive amount of glucose. The liquid glucose was mixed through the soil, using a spatula, after which the respiration rate was measured again for approximately 8 hours. The respiration rate is called the basal respiration before the glucose is added to the samples and SIR respiration after the addition of glucose (Sawada et al., 2009). When using the SIR method, the difference in basal and SIR respiration
rate is an indication of the relative amount of microbial biomass present in the soil. In this report, four hours of basal respiration were compared to four hours of SIR respiration. The values of SIR respiration were not retrieved directly after glucose was added to the soil samples, but approximately 3 hours later. This was done to exclude the disturbed respiration values caused by adding glucose, stirring and aerating the jars.

**Statistical analyses**

The results were statistically analysed using the commercial software package MATLAB R2014b. To discover whether there was a significant difference in the amount of OM, microbial biomass and quality of the soil at the four different heathland communities, the non-parametric Kruskal-Wallis one-way analysis of variance test and multi comparison test were used. The Kruskal-Wallis test was used instead of the one-way ANOVA test as the number of subjects per group differed and as the groups had high variance in standard deviations. In this report, a 0.05 significance level of the statistical tests is chosen. However, before the tests were conducted, all negative values of both methods were removed. This was done as negative values might indicate a measurement error of one of the methods. Subsequently, outliers per soil layer per heathland community were identified using boxplots. These outliers were removed as they might indicate a measurement error as well.

When the FE method was conducted, eight jars were used as a control group. These jars were filled with 0.05 M K$_2$SO$_4$. The TOC was determined for these jars using the TOC analyzer. The average amount of TOC that was measured in the control group was subtracted from all samples in order to compensate for the influence of K$_2$SO$_4$ on the TOC. The SIR method also made use of a control group to detect the influence of glucose on the measurements conducted by the respicond. The control group showed no influence of glucose on the measurements, so these required no additional calculations.

In order to assess the similarities of both methods, a Spearman correlation was conducted as the variance in standard deviation was high over the outcome of biomass per gram dry weight and per gram OM.

All samples are expressed on the same basis in order to make a comparison between the different soil layers per heathland community.
Results
In order to assess the microbial biomass distribution in the topsoil of four differently-aged heathland communities, the OM distribution, microbial biomass per gram dry weight and per gram OM for the FE and SIR method and correlation graphs between the two methods are compared. For all bar graphs, the standard deviation per heathland community per soil layer is included.

Organic matter content based on LOI method
Figure 3 illustrates the OM distribution (in percentage of total dry weight) with soil depth for the four differently-aged heathland communities. For the upper soil layer (0-2 cm), the Middle community stands out with a lower amount of OM compared to the other communities. Furthermore, the figure shows a decline in OM content with soil depth for all heathland communities. No community shows a different distribution.

Table 1 shows the p-values per soil layer for all heathland communities compared based on the LOI method per gram dry weight. For all p-value tables, significant values are underlined. Thus there are significant differences in microbial biomass content between Pioneer and Middle community and Young and Middle community in the 0-2 cm soil layer, Young and Old community for the 2-5 cm soil layer and Middle and Old community for the 5-10 cm soil layer.

Table 1: p-values of the multi comparison test per soil layer for all heathland communities compared based on the results from the LOI method (%)

<table>
<thead>
<tr>
<th>Communities</th>
<th>p-value 0-2 cm</th>
<th>p-value 2-5 cm</th>
<th>p-value 5-10 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>P – Y</td>
<td>0.9993</td>
<td>0.8655</td>
<td>0.9993</td>
</tr>
<tr>
<td>P – M</td>
<td><strong>0.0173</strong></td>
<td><strong>0.5849</strong></td>
<td><strong>0.7129</strong></td>
</tr>
<tr>
<td>P – O</td>
<td>0.7838</td>
<td>0.2751</td>
<td>0.4309</td>
</tr>
<tr>
<td>Y – M</td>
<td><strong>0.0118</strong></td>
<td>0.1729</td>
<td>0.7837</td>
</tr>
<tr>
<td>Y – O</td>
<td>0.7130</td>
<td><strong>0.0496</strong></td>
<td>0.3598</td>
</tr>
<tr>
<td>M – O</td>
<td>0.1878</td>
<td>0.9516</td>
<td><strong>0.0495</strong></td>
</tr>
</tbody>
</table>
**SIR and FE method expressed per gram dry weight**

Figure 4 illustrates the SIR biomass C in µg C/h/g dry weight as measured with the SIR method per heathland community per soil layer. For the upper soil layer, the Middle community shows a lower amount of biomass C compared to the other communities. Furthermore, the figure shows an overall decline of biomass C with soil depth.

![Figure 4: Microbial biomass content (µg C/h/g dry weight) per soil layer of the Pioneer, Young, Middle and Old heathland community based on the SIR method](image)

Figure 5 illustrates the biomass C in mg C/g dry weight as measured with the FE method per heathland community per soil layer. For the upper soil layer, the Middle community shows a lower amount of biomass C compared to the other communities. Furthermore, the figure shows an overall decline of biomass C with soil depth.

![Figure 5: Microbial biomass content (mg C/g dry weight) per soil layer of the Pioneer, Young, Middle and Old heathland community based on the FE method](image)
Table 2 shows the p-values per soil layer for all heathland communities compared based on the SIR method per gram dry weight. There are no significant differences in microbial biomass content between heathland communities per soil layer, even though the Middle community shows a lower amount of biomass C compared to the other communities for the upper soil layer.

Table 2: p-values of the multi comparison test per soil layer for all heathland community compared based on the results from the SIR method (µg C/h/g dry weight)

<table>
<thead>
<tr>
<th>Communities</th>
<th>p-value 0-2 cm</th>
<th>p-value 2-5 cm</th>
<th>p-value 5-10 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>P – Y</td>
<td>0.9614</td>
<td>0.9995</td>
<td>0.7380</td>
</tr>
<tr>
<td>P – M</td>
<td>0.2035</td>
<td>0.8339</td>
<td>0.4443</td>
</tr>
<tr>
<td>P – O</td>
<td>0.8467</td>
<td>0.9788</td>
<td>0.7003</td>
</tr>
<tr>
<td>Y – M</td>
<td>0.0681</td>
<td>0.7748</td>
<td>0.9895</td>
</tr>
<tr>
<td>Y – O</td>
<td>0.5588</td>
<td>0.9583</td>
<td>0.2041</td>
</tr>
<tr>
<td>M – O</td>
<td>0.6628</td>
<td>0.9788</td>
<td>0.0596</td>
</tr>
</tbody>
</table>

Table 3 shows the p-values per soil layer for all heathland communities compared based on the FE method per gram dry weight. There are significant differences in microbial biomass content between the Pioneer and Middle community and Young and Middle community for the upper soil layer (0-2 cm), between the Young and Middle community and Young and Old community for the next soil layer (2-5 cm) and between the Young and Old community for the 5-10 cm soil layer.

Table 3: p-values of the multi comparison test per soil layer for all heathland community compared based on the results from the FE method (µg C/g dry weight)

<table>
<thead>
<tr>
<th>Communities</th>
<th>p-value 0-2 cm</th>
<th>p-value 2-5 cm</th>
<th>p-value 5-10 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>P – Y</td>
<td>0.9977</td>
<td>0.5327</td>
<td>0.3451</td>
</tr>
<tr>
<td>P – M</td>
<td>0.0035</td>
<td>0.2951</td>
<td>0.9993</td>
</tr>
<tr>
<td>P – O</td>
<td>0.1693</td>
<td>0.1219</td>
<td>0.1146</td>
</tr>
<tr>
<td>Y – M</td>
<td>0.0031</td>
<td>0.0103</td>
<td>0.4181</td>
</tr>
<tr>
<td>Y – O</td>
<td>0.1397</td>
<td>0.0022</td>
<td>0.0008</td>
</tr>
<tr>
<td>M – O</td>
<td>0.6190</td>
<td>0.9698</td>
<td>0.0862</td>
</tr>
</tbody>
</table>
Figure 6 illustrates the correlation between the outcomes of the FE and SIR method per gram dry weight. The correlation coefficient is 0.9650, so there is a strong positive correlation between both methods per gram dry weight. This is strengthened with the p-value of 0, which implies that there is a correlation between the two methods as the correlation coefficient is significantly different from zero.

Figure 6: Correlation of the FE and SIR method (/g dry weight)
SIR and FE method expressed per gram OM

Figure 7 illustrates the biomass C in µg C/h/g OM as measured with the SIR method per heathland community per soil layer. The figure shows an overall decline in microbial biomass per gram OM with soil depth, except for the Young community which shows a higher amount of microbial biomass in the 5-10 cm soil layer compared to the 2-5 cm soil layer.

![Figure 7](image)

Figure 7: Microbial biomass content (µg C/h/g OM) per soil layer of the Pioneer, Young, Middle and Old heathland community based on the SIR method

Figure 8 illustrates the biomass C in mg C/g OM as measured with the FE method per heathland community per soil layer. The figure shows a overall steadiness of biomass C with soil depth. The biomass C per gram OM only declines for the Old community in the 5-10 cm soil layer.

![Figure 8](image)

Figure 8: Microbial biomass content (mg C/ g OM) per soil layer of the Pioneer, Young, Middle and Old heathland community based on the FE method
Table 5 shows the p-values per soil layer for all heathland communities compared based on the SIR method per gram OM. There are no significant differences in microbial biomass between the heathland communities per soil layer.

<table>
<thead>
<tr>
<th>Communities</th>
<th>p-value 0-2 cm</th>
<th>p-value 2-5 cm</th>
<th>p-value 5-10 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>P – Y</td>
<td>0.9919</td>
<td>0.8730</td>
<td>0.6311</td>
</tr>
<tr>
<td>P – M</td>
<td>0.9910</td>
<td>0.9523</td>
<td>0.2771</td>
</tr>
<tr>
<td>P – O</td>
<td>0.8058</td>
<td>0.9800</td>
<td>0.7381</td>
</tr>
<tr>
<td>Y – M</td>
<td>0.9880</td>
<td>0.9978</td>
<td>0.9997</td>
</tr>
<tr>
<td>Y – O</td>
<td>0.6370</td>
<td>0.0531</td>
<td>0.9284</td>
</tr>
<tr>
<td>M – O</td>
<td>0.8268</td>
<td>0.9716</td>
<td>0.8861</td>
</tr>
</tbody>
</table>

Table 6 shows the p-values per soil layer for all heathland communities compared based on the FE method per gram OM. There are significant differences between microbial biomass content of the Pioneer and Old community and Young and Old community for the lowest soil layer (5-10 cm).

<table>
<thead>
<tr>
<th>Communities</th>
<th>p-value 0-2 cm</th>
<th>p-value 2-5 cm</th>
<th>p-value 5-10 cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>P – Y</td>
<td>0.8504</td>
<td>0.7838</td>
<td>0.7913</td>
</tr>
<tr>
<td>P – M</td>
<td>0.7368</td>
<td>0.9145</td>
<td>0.3919</td>
</tr>
<tr>
<td>P – O</td>
<td>0.7119</td>
<td>0.9282</td>
<td>0.0371</td>
</tr>
<tr>
<td>Y – M</td>
<td>0.2590</td>
<td>0.3831</td>
<td>0.0535</td>
</tr>
<tr>
<td>Y – O</td>
<td>0.2571</td>
<td>0.4068</td>
<td>0.0015</td>
</tr>
<tr>
<td>M – O</td>
<td>0.9997</td>
<td>1.0000</td>
<td>0.6548</td>
</tr>
</tbody>
</table>
Figure 9 illustrates the correlation between the outcomes of the FE and SIR method per gram OM. The correlation coefficient is $-0.1259$, so there is a weak negative correlation between both method per gram OM. This is strengthened with the $p$-value of $0.69976$, which implies that there is no correlation between the two methods as the correlation coefficient is not significantly different from zero.

The MATLAB 2014 scripts and data input for each bar graph figure and $p$-value table can be found in appendix B.
Discussion

Microbial biomass content
The OM content illustrated in figure 3 shows a decline with soil depth for all heathlands communities. This distribution is similar for the amount of microbial biomass per gram dry weight resulting from the SIR and FE method, as seen in figure 4 and 5. This outcome is supported by the results from the study by Murphy et al. (1998). As stated in the introduction, Murphy et al. (1998) reported that the highest quantity of microbial biomass is mostly found in the soil layer with the highest content of OM and that this is often within the first 5 cm of the soil. Furthermore, the Middle community shows a significantly lower OM content within the first 2 cm of the topsoil compared to the Pioneer and Young community. This significant difference between these communities is also indicated by the FE method in table 3. The Middle community has been ploughed a couple of years ago. These results are similar to the outcome of the study by Woods (1989), as stated in the introduction. Woods (1989) discovered that the amount of microbial biomass in the surface layer of the undisturbed soil was significantly higher than the disturbed soil. The SIR method does not show significant differences between these heathland communities, as can be observed in table 2, and are subsequently not similar to the outcome of Woods (1989). However, the correlation between the FE and SIR methods is high (p= 0), as seen in figure 6. This indicates that the results of both methods have a similar distribution.

When the amount of microbial biomass is expressed per gram dry weight, multiple influences could explain significant differences, such as difference in the amount or quality of OM. However, when it is expressed per gram OM, only the quality of OM can explain significant differences. Therefore, both methods are expressed per gram OM as well.

Soil quality
The SIR and FE method show a different distribution when the microbial biomass is expressed per gram OM, as seen in figure 7 and 8. This is shown with the low correlation (p= 0.6997) between the methods in figure 9. The FE method indicates significant differences between the Pioneer and Old community and Middle and Old community for the 5-10 cm soil layer, as can be observed in table 6. These significant differences are not shown by the SIR method in table 5. Furthermore, the FE methods does not show a decline in OM quality with soil depth, except for the Old community at 5-10 cm. This decline of OM quality with soil depth is illustrated by the SIR method.

The difference in OM quality indicated by the FE method in the 5-10 cm soil layer could be caused by a higher amount of lignin present in the soil layer of the Old community heathland, as stated in the introduction (Austin & Ballaré, 2010). The easily degradable OM of the Old community will have been mineralized first, but with increasing soil depth less easily degradable OM is present resulting in a higher lignin content. As the Pioneer and Young community contains a lower amount of lignin, there is no significant decline in OM quality with soil depth (Gimingham, 1985).

The difference in OM quality indicated by the FE method in the 5-10 cm soil layer could also be caused by a lower amount of root exudation of the Old community compared to the Pioneer and Young community. Root exudation is the secretion of easily degradable substrates by plant roots in the rhizosphere (Walker et al., 2003). When Calluna is in the degeneration phase, it is possible that the exudation is declining, resulting in less microbial biomass in the specific soil layer.
The difference in distribution between both methods could be explained if the SIR method indicates an overall lower amount of microbial biomass compared to the FE method with soil depth, as both are divided by the OM content of the samples. However, the results of both methods are not expressed in the same units so a direct comparison is not possible. Therefore, the emphasis is on relative differences. It would have been possible to recalculate the SIR biomass C to mg C/g dry weight or OM, using the following formula: SIR-biomass C (μg C g⁻¹ soil) = SIR (μL CO₂ g⁻¹ soil h⁻¹) × 40.04 + 0.37 (Anderson & Domsch, 1978). However, for this formula only the maximum initial respiration after adding glucose is used, while for this report the difference in basal and SIR respiration is used as an indication of microbial biomass. Additionally, multiple recalculations are required before the formula can be used, such as correcting the respiration rates by a factor of 0.843 and correcting the effects of temperature on the respiration rate, as the formula was created under different temperature conditions (22°C instead of 20°C, which is used in this report) (Sawada et al., 2009). However, the correction factor for respiration rates is not found in all articles. Therefore, recalculating to SIR biomass C in mg C/g dry weight or OM may result in substantial errors when not all parameters are known.

First of all, for the FE method, it was assumed that the total microbial biomass of the fumigated samples is killed within the 24 hours and that only microbial biomass carbon is dissolved in the extraction (Jenkinson et al., 2004). These assumptions could either underestimate or overestimate the amount of microbial biomass in topsoil. However, the advantage of this technique, is that it can be applied to acid soils (Vance et al., 1987).

Secondly, for the SIR method, it was assumed that all microorganisms use the glucose as an energy source (Sawada et al., 2009). However, the SIR method could indicate a lower amount of microbial biomass compared to the FE method as the former only includes bacteria in the first hours of the experiment. Behera and Wagner (1974) investigated the microbial growth rate in soils, after the addition of glucose. They discovered that the bacteria were responsible for the increase in CO₂ emissions from the soil during the first two days. Only after the amount of bacteria declined, the amount of fungi started to increase. Due to this, the amount of microbial biomass indicated by the SIR method might be underestimated. Especially as acid soils usually contain a higher fungal-to-bacteria ratio than pH neutral soils (Blagodatskaya & Anderson, 1998).

Thirdly, the FE method includes all microorganisms in a sample, even though the microorganisms are inactive (Klein et al., 1998). These microorganisms do no longer contribute to respiration and are therefore not included in the SIR method and therefore underestimates the amount of microbial biomass as well.

Last, the glucose that was added to the samples in the respicond might not have been mixed homogeneously. However, this is important for obtaining reliable results from the SIR method (Lin & Brookes, 1999). There were only 60 seconds for adding the glucose and mixing it through the soil. As a result, not all the microorganisms might have received a similar amount of glucose, which underestimates the amount of microorganisms in the soil measured with the SIR method.
Results related to hypothesis and relevance
Both methods do not support the hypothesis as stated in the introduction. It was hypothesised that the highest concentration of microbial biomass would be located in the Pioneer and Young community for all soil layers compared to the Middle and Old community as well as the highest soil quality. The SIR method does not indicate any significant differences between these communities and the FE method only indicates several significant differences between these communities. For the amount of microbial biomass expressed per gram dry weight, the significant differences are between the Pioneer and Middle community and Young and Middle community for the 0-2 cm soil layer, between the Young and Middle community and Young and Old community for the 2-5 cm soil layer and between the Young and Old community for the 5-10 cm soil layer. For the amount of microbial biomass expressed per gram OM, the significant differences are between the Pioneer and Old community and Young and Old community for the 5-10 cm soil layer.

As the Old community has a significantly lower soil quality compared to the Pioneer and Young community for the 5-10 cm soil layer based on the FE method, the microbial efficiency in this soil layer is lower as well (Frey et al., 2013). Subsequently, more OM is converted into CO$_2$ instead of microbial biomass. Therefore, it could be recommended to change management practices and cut heathland before it enters the degeneration phase. However, the SIR method does not indicate this difference, so a third method for measuring microbial biomass could be applied.

Suggestions for future research
Research done by West et al. (1986) indicated the preference of using three methods for measuring the amount of microbial biomass in soil. Besides the FE and SIR technique, the fumigation incubation and ATP extraction methods can be used for measuring microbial biomass (Martens, 1995). The fumigation incubation method uses chloroform to kill the microorganisms in a sample, similar to the FE method. The sample is then incubated for 10 days. A control group is not fumigated but left for 10 days of incubation as well. After the incubation, the difference in amount of CO$_2$ released from the soils is calculated, which is an indication for microbial biomass. However, the fumigation incubation method does not properly measure the microbial biomass in acid soils. The ATP extraction method measures the amount of microbial biomass by the quantification of ATP, which is present in microorganisms. The advantages of measuring ATP is that it degrades rapidly in dead organisms and when it is free in the soil. Furthermore, it is present in all microorganisms. However, it cannot be extracted completely and it easily absorbs to soil particles after extraction. This method can be applied to acid soils (Martens, 1995). The currently used methods with which the ATP extraction method shows the highest correlation and lowest p-value expressed both per gram dry weight and OM, could suggest that this method measured the total microbial biomass more precisely.

This research identified the difference in amount of microbial biomass and soil quality of four differently-aged heathland communities. However, no distinction was made between microorganisms with regard to bacteria and fungi. Previous research by Eiland et al. (2001) and De Vries et al. (2006) showed that soils with a low C/N ratio were dominated by bacteria, while soils with a high C/N ratio were dominated by fungi. Fungi and bacteria both have an influence on the soil dynamics. For example, due to the difference in C/N ratio of fungi and bacteria, 10 and 4 respectively, the N mineralisation rate caused by fungivores and bacterivores will be lower in a fungi dominated environment compared to a bacteria dominated environment (De Vries et al., 2006). As the ratio of fungi to bacteria biomass influences the soil dynamics differently, further research could
investigate whether this ratio differs between the four differently-aged heathland communities at the research site in Oldebroek. Subsequently, when the composition of the microbial biomass is known, the difference in outcome of the FE and SIR might be better explained as SIR is less sensible for detecting fungi biomass in the first hours after the addition of glucose (Behera & Wagner, 1974).

Furthermore, at the start of this research, it was chosen to divide the upper 10 cm of the soil into three parts regardless of the difference in chemical properties of the topsoil between all sample locations. As a result, no difference was made based on the distribution of horizons and different horizons might have been compared. Additionally, within one heathland community, there might also have been great variety in the distribution of horizons. This might explain the high standard deviation in the 0-2 and 2-5 cm of the topsoil as certain samples might have had a thicker organic horizon than other samples within the sample community. For future research, it might be decided to either only compare the same horizons between heathland communities or to choose a different partition of the first 10 cm. For example a centimetre-scale partitioning of the top soil might be a more suitable sampling strategy, as the highest standard deviations are found in the 0-2 cm and 2-5 cm soil layers.

For this research, the samples were searched through to exclude stones and roots by hand. This method might not have been precise enough, and gravel might have been included in samples used for the FE and SIR method. This might have resulted in a lower amount of microbial biomass for both methods than would be the case otherwise. Therefore, it is suggested to carefully sieve the samples in future research.

Furthermore, the moisture content was equalized per soil layer per heathland community in order to minimize the influence of moisture content on the mineralization rate of microorganisms. However, to be able to compare all heathland communities per soil layer, the moisture content should have been equalized per soil layer for all heathland communities. Tietema et al. (1992) discovered that the CO$_2$ production increased with increasing moisture content up to approximately 170%. In appendix C, the moisture contents of all samples per soil layer per heathland community can be found. For none of the soil layers, the moisture content of all heathland communities is above 170%. However, the moisture content does vary substantially within each soil layer. This might have influenced the results of the SIR method as the rate of mineralization depends on the moisture content (Curtin et al., 2012). Future research should equalize water content per soil layer for all heathland communities.
Conclusion
This research was conducted to investigate the effects of four differently-aged heathland communities on the vertical distribution of microbial biomass in the topsoil. The results of the FE and SIR method indicated different effects. The FE method showed several significant differences when expressed per gram dry weight and per gram OM, whereas the SIR method did not indicate any significant differences either expressed per gram dry weight or gram OM. Therefore, it can be concluded for the FE method that the four differently-aged heathland communities have an effect on the amount of microbial biomass and soil quality with soil depth per soil layer. Though, for the SIR method it can be concluded that the four differently-aged heathland communities have no effect on the amount of microbial biomass and soil quality with soil depth per soil layer.

The hypothesis stated that the Pioneer and Young community would contain the highest amount of microbial biomass and highest quality of soil for all soil layers compared to the Middle and Old community. As said before, the SIR method indicated no significant differences either based per gram dry weight or OM. Additionally, even though the FE method indicated significant differences between certain soil layers, it did not show the consistency of a higher amount of microbial biomass or higher soil quality for all soil layers for the Pioneer and Young community compared to the Middle and Old community. Therefore, the hypothesis is not accepted.

The results of the SIR method might have been underestimated. However, this cannot be known for certain so it is recommended to perform a third method for measuring the microbial biomass, such as the ATP extraction method.
Acknowledgements
I would like to express my sincere gratitude to the following people for helping me with my bachelor thesis:

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Henk Pieter Sterk, for his support during fieldwork, guidance with interpreting the results and providing feedback on my thesis.

Marleen van Dusseldorp, for making it so much fun to collect samples and prepare and analyze them in the laboratory.

And last but not least, Jaleesa Schaap, for all the fun and companionship during fieldwork.
References


Appendix

A: Mean values moisture content and LOI

Table 1: mean values of gram field moisture, gram dry weight, percentage moisture content and LOI per gram and percentage

<table>
<thead>
<tr>
<th>Sample</th>
<th>gram field moisture</th>
<th>gram dry weight</th>
<th>moisture content (%)</th>
<th>LOI (g)</th>
<th>LOI (%)</th>
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</table>
### B: Matlab script and data input

**Organic matter distribution**

Table 2: Organic matter distribution (%) per soil layer of the Pioneer, Young, Middle and Old heathland community – data input MATLAB

<table>
<thead>
<tr>
<th>Soil depth (cm)</th>
<th>0-2</th>
<th>2-5</th>
<th>5-10</th>
</tr>
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<tr>
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<td>Middle</td>
</tr>
<tr>
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<td>21.29</td>
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<td>51.18</td>
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<tr>
<td>mean</td>
<td>49.06</td>
<td>50.19</td>
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</table>

%MATLAB SCRIPT ORGANIC MATTER DISTRIBUTION
%FIEKE VLAAR - 10467165

%CLEAR WORKING SPACE%
close all

clear

clc

%LOADING DATA%
[P02 J02 M02 O02 P25 J25, M25 O25 P510 J510 M510 O510] =textread('OM.txt', '%f%f%f%f%f%f%f%f%f', 'delimiter', 'tab', 'headerlines', 2);

%ASSIGNING DATA TO VARIABLES%
Pioneer02 = P02(1:6);
Pioneer25 = P25(1:6);
Pioneer510 = P510(1:6);
Jong02 = J02(1:6);
Jong25 = J25(1:6);
Jong510 = J510(1:6);
Middel02 = M02(1:6);
Middel25 = M25(1:6);
Middel510 = M510(1:6);
Oud02 = O02(1:6);
Oud25 = O25(1:6);
Oud510 = 0510(1:6);

% CREATING GROUPS FOR CONDUCTING THE KRUSKALWALLIS TEST%
Laag02 = [Pioneer02; Jong02; Middel02; Oud02];
Laag25 = [Pioneer25; Jong25; Middel25; Oud25];
Laag510 = [Pioneer510; Jong510; Middel510; Oud510];
Group = [ones(6,1); (ones(6,1)*2); (ones(6,1)*3); (ones(6,1)*4)];

% CONDUCTING THE KRUSKALWALLIS TEST%
[p, tbl, stats1]=kruskalwallis(Laag02,Group);
[p, tbl, stats2]=kruskalwallis(Laag25,Group);
[p, tbl, stats3]=kruskalwallis(Laag510,Group);

% CONDUCTING THE MULTICOMPARISON TEST%
multcompare(stats1)
multcompare(stats2)
multcompare(stats3)

% CALCULATING THE STANDARD DEVIATIONS%
std1= std(Pioneer02);
std2=std(Pioneer25);
std3=std(Pioneer510);
std4=std(Jong02);
std5=std(Jong25);
std6=std(Jong510);
std7=std(Middel02);
std8=std(Middel25);
std9=std(Middel510);
std10=std(Oud02);
std11=std(Oud25);
std12=std(Oud510);

% CREATING VARIABLES FOR BAR GRAPH WITH STANDARD DEVIATION%
std_fumigatie=[std1,std2,std3,std4,std5,std6,std7,std8,std9,std10,std11,std12];
y= [P02(7) P25(7) P510(7); J02(7) J25(7) J510(7); M02(7) M25(7) M510(7); O02(7) O25(7) O510(7)];

% CREATING BAR GRAPH WITH STANDARD DEVIATION%
bb=bar(y'); hold all
data=repmat([1:4]'*ones(3,1)',1,1);
for i = 1:3;
    j = 1:4;
    x = -0.5 + i + 1/5 * j;
    errorbar(x, y(j,i), std_fumigatie(j,i), '.');
end

% COMPLETING THE BAR GRAPH WITH LABELS, TITLE AND LEGEND%
labels = {'0-2','2-5','5-10'};
set(gca, 'XTick', 1:12, 'XTickLabel', labels);
title 'Organic matter content based on the LOI method'
ylabel 'OM (%)'
xlabel 'Soil depth (cm)'
ln={‘Pioneer’,’Young’,’Middle’,’Old’};
legend(ln);
SIR biomass C per gram dry weight

Table 3: SIR biomass C (µg C/h/g dry weight) per soil layer of the Pioneer, Young, Middle and Old heathland community – data input MATLAB

<table>
<thead>
<tr>
<th>Soil depth (cm)</th>
<th>0-2</th>
<th>2-5</th>
<th>5-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Pioneer</td>
<td>Young</td>
<td>Middle</td>
</tr>
<tr>
<td>Replica 1</td>
<td>14.62</td>
<td>19.52</td>
<td>3.28</td>
</tr>
<tr>
<td>2</td>
<td>12.99</td>
<td>14.34</td>
<td>6.77</td>
</tr>
<tr>
<td>3</td>
<td>14.91</td>
<td>33.06</td>
<td>4.23</td>
</tr>
<tr>
<td>4</td>
<td>5.56</td>
<td>33.56</td>
<td>9.53</td>
</tr>
<tr>
<td>5</td>
<td>18.77</td>
<td>17.26</td>
<td>3.07</td>
</tr>
<tr>
<td>6</td>
<td>27.03</td>
<td>2.98</td>
<td>5.36</td>
</tr>
<tr>
<td>mean</td>
<td>15.65</td>
<td>20.12</td>
<td>5.37</td>
</tr>
</tbody>
</table>

%MATLAB SCRIPT SUBSTRATE INDUCED RESPIRATION METHOD (SIR BIOMASS C PER GRAM DRY WEIGHT)
%FIEKE VLAAR - 10467165

%CLEAR WORKING SPACE%
close all
clear
clc

%LOADING DATA%
[P02 J02 M02 O02 P25 J25, M25 O25 P510 J510 M510 O510] =textread('RespicondOmrekeningVerschil.txt','%f%f%f%f%f%f%f%f%f%f%f.','delimiter','tab','headerlines',2);

%ASSIGNING DATA TO VARIABLES%
Pioneer02 = P02(1:6);
Pioneer25 = P25(1:5);
Pioneer510 = P510(1:6);
Jong02 = J02(1:6);
Jong25 = J25(1:5);
Jong510 = J510(1:4);
Middel02 = M02(1:6);
Middel25 = M25(1:5);
Middel510 = M510(1:6);
Oud02 = O02(1:6);
Oud25 = O25(1:4);
Oud510 = O510(1:5);

%CHECKING DATA FOR OUTLIERS%
boxplot(Pioneer02);
boxplot(Pioneer02);
boxplot(Pioneer25);
boxplot(Pioneer510);
boxplot(Jong02);
boxplot(Jong25);
boxplot(Jong510);
boxplot(Middel02);
boxplot(Middel25);
boxplot(Middel510);
boxplot(Oud02);
boxplot(Oud25);
boxplot(Oud510);

%CREATING GROUPS FOR CONDUCTING THE KRUSKALWALLIS TEST%
Laag02 = [Pioneer02; Jong02; Middel02; Oud02];
Laag25 = [Pioneer25; Jong25; Middel25; Oud25];
Laag510 = [Pioneer510; Jong510; Middel510; Oud510];
Group1 = [ones(6,1); (ones(6,1)*2); (ones(6,1)*3); (ones(6,1)*4)];
Group2 = [ones(5,1); (ones(5,1)*2); (ones(5,1)*3); (ones(4,1)*4)];
Group3 = [ones(6,1); (ones(4,1)*2); (ones(6,1)*3); (ones(5,1)*4)];

%CONDUCTING THE KRUSKALWALLIS TEST%
[p, tbl, stats1]=kruskalwallis(Laag02,Group1);
[p, tbl, stats2]=kruskalwallis(Laag25,Group2);
[p, tbl, stats3]=kruskalwallis(Laag510,Group3);

%CONDUCTING THE MULTICOMPARISON TEST%
multcompare(stats1)
multcompare(stats2)
multcompare(stats3)

%CALCULATING THE STANDARD DEVIATIONS%
std1= std(Pioneer02);
std2=std(Pioneer25);
std3=std(Pioneer510);
std4=std(Jong02);
std5=std(Jong25);
std6=std(Jong510);
std7=std(Middel02);
std8=std(Middel25);
std9=std(Middel510);
std10=std(Oud02);
std11=std(Oud25);
std12=std(Oud510);

%CREATING VARIABLES FOR BAR GRAPH WITH STANDARD DEVIATION%
std_fumigatie=[std1,std2,std3,std4,std5,std6,std7,std8,std9,std10,std11,std12];
y=[P02(7) P25(7) P510(7); J02(7) J25(7) J510(7); M02(7) M25(7) M510(7); O02(7) O25(7) O510(7)];

%CREATING BAR GRAPH WITH STANDARD DEVIATION%
bb=bar(y'); hold all
data=repmat([1:4]'*ones(3,1)',1,1);
for i = 1:3;
    j = 1:i;
    x = -0.5 + i + 1/5 * j;
    errorbar(x, y(j,i), std_fumigatie(j,i), '.');
end
%COMPLETING THE BAR GRAPH WITH LABELS, TITLE AND LEGEND%
labels = {'0-2','2-5','5-10'};
set(gca, 'Xtick', 1:12, 'XtickLabel', labels)
title 'Microbial biomass content per gram dry weight based on the SIR method'
ylabel 'SIR biomass C (µg C/h/g dry weight)'
xlabel 'Soil depth (cm)'
ln={‘Pioneer’,‘Young’,‘Middle’,‘Old’};
legend(ln);

SIR biomass C per gram OM

Table 4: SIR biomass C (µg C/h/g OM) per soil layer of the Pioneer, Young, Middle and Old heathland community - data input MATLAB

<table>
<thead>
<tr>
<th>Soil depth (cm)</th>
<th>Group</th>
<th>0-2</th>
<th>2-5</th>
<th>5-10</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pioneer</td>
<td>Young</td>
<td>Middle</td>
<td>Old</td>
</tr>
<tr>
<td>1</td>
<td>21.88</td>
<td>39.69</td>
<td>15.47</td>
<td>35.55</td>
</tr>
<tr>
<td>3</td>
<td>45.79</td>
<td>44.16</td>
<td>33.46</td>
<td>33.44</td>
</tr>
<tr>
<td>4</td>
<td>9.84</td>
<td>69.45</td>
<td>50.98</td>
<td>42.71</td>
</tr>
<tr>
<td>5</td>
<td>43.10</td>
<td>41.59</td>
<td>29.36</td>
<td>30.00</td>
</tr>
<tr>
<td>6</td>
<td>61.30</td>
<td>8.78</td>
<td>39.21</td>
<td>1.57</td>
</tr>
<tr>
<td>mean</td>
<td>34.55</td>
<td>38.44</td>
<td>34.71</td>
<td>25.77</td>
</tr>
</tbody>
</table>

%MATLAB SCRIPT SUBSTRATE INDUCED RESPIRATION METHOD (SIR BIOMASS C PER GRAM OM)
%FIEKE VLAAR - 10467165

%CLEAR WORKING SPACE%
close all

clear

clc

%LOADING DATA%
[P02 J02 M02 O02 P25 J25, M25 O25 P510 J510 M510 O510] =textread('RespicondOmrekeningOM.txt','%f%f%f%f%f%f%f%f%f%f','%delimiter','','tab','','headerlines',2);
%CHECKING DATA FOR OUTLIERS%
boxplot(Pioneer02);
boxplot(Pioneer02);
boxplot(Pioneer02);
boxplot(Pioneer510);
boxplot(Jong02);
boxplot(Jong02);
boxplot(Jong510);
boxplot(Middel02);
boxplot(Middel02);
boxplot(Middel125);
boxplot(Middel125);
boxplot(Oud02);
boxplot(Oud02);
boxplot(Oud510);
boxplot(Oud510);

%CREATING GROUPS FOR CONDUCTING THE KRUSKAL-WALLIS TEST%
Laag02 = [Pioneer02; Jong02; Middel02; Oud02];
Laag25 = [Pioneer25; Jong25; Middel25; Oud25];
Laag510 = [Pioneer510; Jong510; Middel510; Oud510];
Group1 = [ones(6,1); (ones(6,1)*2); (ones(6,1)*3); (ones(6,1)*4)];
Group2 = [ones(5,1); (ones(6,1)*2); (ones(4,1)*3); (ones(4,1)*4)];
Group3 = [ones(5,1); (ones(5,1)*2); (ones(6,1)*3); (ones(5,1)*4)];

%CONDUCTING THE KRUSKAL-WALLIS TEST%
[p, tbl, stats1]=kruskalwallis(Laag02,Group1);
[p, tbl, stats2]=kruskalwallis(Laag25,Group2);
[p, tbl, stats3]=kruskalwallis(Laag510,Group3);

%CONDUCTING THE MULTICOMPARISON TEST%
multcompare(stats1)
multcompare(stats2)
multcompare(stats3)

%CALCULATING THE STANDARD DEVIATIONS%
std1= std(Pioneer02);
std2=std(Pioneer25);
std3=std(Pioneer510);
std4=std(Jong02);
std5=std(Jong25);
std6=std(Jong510);
std7=std(Middel02);
std8=std(Middel25);
std9=std(Middel510);
std10=std(Oud02);
std11=std(Oud25);
std12=std(Oud510);

%CREATING VARIABLES FOR BAR GRAPH WITH STANDARD DEVIATION%
std_fumigatie=[std1, std2, std3; std4, std5, std6; std7, std8, std9; std10, std11, std12];
y = [P02(7) P25(7) P510(7); J02(7) J25(7) J510(7); M02(7) M25(7) M510(7); O02(7) O25(7) O510(7)];

figure
%CREATING BAR GRAPH WITH STANDARD DEVIATION%
bb=bar(y'); hold all
data=repmat([1:4]'*ones(3,1)',1,1);
for i = 1:3;
    j = 1:4;
    x = -0.5 + i + 1/5 * j;
    errorbar(x, y(j,i), std_fumigatie(j,i), '.');
end

%COMPLETING THE BAR GRAPH WITH LABELS, TITLE AND LEGEND%
labels = {'0-2','2-5','5-10'};
set(gca, 'XTick', 1:12, 'XTickLabel', labels);
title 'Microbial biomass content per gram OM based on the SIR method'
ylabel 'SIR biomass C (µg C/h/g OM)'
xlabel 'Soil depth (cm)'
ln=['Pioneer','Young','Middle','Old'];
legend(ln)

FE biomass C per gram dry weight

Table 5: FE biomass C (mg C/ g dry weight) per soil layer of the Pioneer, Young, Middle and Old heathland community - data input MATLAB

<table>
<thead>
<tr>
<th>Soil depth (cm)</th>
<th>0-2</th>
<th>2-5</th>
<th>5-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>Pioneer</td>
<td>Young</td>
<td>Middle</td>
</tr>
<tr>
<td>Replica 1</td>
<td>4.11</td>
<td>3.20</td>
<td>1.09</td>
</tr>
<tr>
<td>2</td>
<td>3.81</td>
<td>2.55</td>
<td>1.03</td>
</tr>
<tr>
<td>3</td>
<td>3.05</td>
<td>3.73</td>
<td>0.72</td>
</tr>
<tr>
<td>4</td>
<td>2.68</td>
<td>3.40</td>
<td>1.12</td>
</tr>
<tr>
<td>5</td>
<td>2.43</td>
<td>3.42</td>
<td>0.67</td>
</tr>
<tr>
<td>6</td>
<td>3.19</td>
<td>-</td>
<td>0.67</td>
</tr>
<tr>
<td>mean</td>
<td>3.21</td>
<td>3.26</td>
<td>0.88</td>
</tr>
</tbody>
</table>

%MATLAB SCRIPT FUMIGATION EXTRACTION METHOD (FE BIOMASS C PER GRAM DRY WEIGHT)
%FIEKE VLAAR - 10467165

%CLEAR WORKING SPACE%
close all
clear
clc
%LOADING DATA%
=textread('FumigatieOmrekening.txt','%f%f%f%f%f%f%f%f%f%f%f%f
delimiter','tab','headerlines',2);

%ASSIGNING DATA TO VARIABLES%
Pioneer02 = P02(1:6);
Pioneer25 = P25(1:6);
Pioneer510 = P510(1:6);
Jong02 = J02(1:5);
Jong25 = J25(1:6);
Jong510 = J510(1:6);
Middel02 = M02(1:6);
Middel25 = M25(1:6);
Middel510 = M510(1:6);
Oud02 = O02(1:5);
Oud25 = O25(1:6);
Oud510 = O510(1:5);

%CHECKING DATA FOR OUTLIERS%
boxplot(Pioneer02);
boxplot(Pioneer25);
boxplot(Pioneer510);
boxplot(Jong02);
boxplot(Jong25);
boxplot(Jong510);
boxplot(Middel02);
boxplot(Middel25);
boxplot(Middel510);
boxplot(Oud02);
boxplot(Oud25);
boxplot(Oud510);

%CREATING GROUPS FOR CONDUCTING THE KRUSKALWALLIS TEST%
Laag02 = [Pioneer02;Jong02;Middel02;Oud02];
Laag25 = [Pioneer25;Jong25;Middel25;Oud25];
Laag510 = [Pioneer510;Jong510;Middel510;Oud510];
Group1 = [ones(6,1);(ones(5,1)*2);(ones(6,1)*3);(ones(5,1)*4)];
Group2 = [ones(6,1);(ones(6,1)*2);(ones(6,1)*3);(ones(6,1)*4)];
Group3 = [ones(6,1);(ones(6,1)*2);(ones(6,1)*3);(ones(5,1)*4)];

%CONDUCTING THE KRUSKALWALLIS TEST%
[p, tbl, stats1]=kruskalwallis(Laag02,Group1);
[p, tbl, stats2]=kruskalwallis(Laag25,Group2);
[p, tbl, stats3]=kruskalwallis(Laag510,Group3);

%CONDUCTING THE MULTICOMPARISON TEST%
multcompare(stats1)
multcompare(stats2)
multcompare(stats3)

%CALCULATING THE STANDARD DEVIATIONS%
std1= std(Pioneer02);
std2=std(Pioneer25);
std3=std(Pioneer510);
std4=std(Jong02);
std5=std(Jong25);
std6=std(Jong510);
%CREATING VARIABLES FOR BAR GRAPH WITH STANDARD DEVIATION%
std7=std(Middel02);
std8=std(Middel25);
std9=std(Middel510);
std10=std(Oud02);
std11=std(Oud25);
std12=std(Oud510);

%CREATING VARIABLES FOR BAR GRAPH WITH STANDARD DEVIATION%
std_fumigatie=[std1,std2,std3;std4,std5,std6;std7,std8,std9;std10,std11,std12];
y= [P02(7) P25(7) P510(7); J02(7) J25(7) J510(7); M02(7) M25(7) M510(7); O02(7) O25(7) O510(7)];

%CREATING BAR GRAPH WITH STANDARD DEVIATION%
bb=bar(y'); hold all
data=repmat([1:4]'*ones(3,1)',1,1);
for i = 1:3;
    j = 1:4;
    x = -0.5 + i + 1/5 * j;
    errorbar(x, y(j,i), std_fumigatie(j,i), '.');
end

%COMPLETING THE BAR GRAPH WITH LABELS, TITLE AND LEGEND%
labels = {'0-2','2-5','5-10'};
set(gca, 'XTick', 1:12, 'XTickLabel', labels);
title 'Microbial biomass content per gram dry weight based on the FE method''
ylabel 'FE biomass C (mg C/g dry weight)'
xlabel 'Soil depth (cm)'
ln={'Pioneer','Young','Middle','Old'};
legend(ln)

FE biomass C per gram OM

Table 6: FE biomass C (mg C/ g OM) per soil layer of the Pioneer, Young, Middle and Old heathland community - data input MATLAB

<table>
<thead>
<tr>
<th>Soil depth (cm)</th>
<th>0-2</th>
<th>2-5</th>
<th>5-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
<td>2</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Replica 1</td>
<td>6.16</td>
<td>4.21</td>
<td>5.69</td>
</tr>
<tr>
<td></td>
<td>6.52</td>
<td>4.76</td>
<td>6.79</td>
</tr>
<tr>
<td></td>
<td>5.12</td>
<td>5.92</td>
<td>7.70</td>
</tr>
<tr>
<td></td>
<td>7.80</td>
<td>4.70</td>
<td>7.70</td>
</tr>
<tr>
<td></td>
<td>4.21</td>
<td>6.24</td>
<td>5.55</td>
</tr>
<tr>
<td></td>
<td>6.42</td>
<td>7.41</td>
<td>7.54</td>
</tr>
<tr>
<td></td>
<td>6.60</td>
<td>9.04</td>
<td>6.14</td>
</tr>
<tr>
<td></td>
<td>6.97</td>
<td>6.14</td>
<td>5.56</td>
</tr>
<tr>
<td></td>
<td>5.84</td>
<td>6.66</td>
<td>4.87</td>
</tr>
<tr>
<td></td>
<td>5.55</td>
<td>5.38</td>
<td>2.39</td>
</tr>
<tr>
<td></td>
<td>5.94</td>
<td>6.33</td>
<td>4.95</td>
</tr>
<tr>
<td></td>
<td>5.65</td>
<td>8.06</td>
<td>5.54</td>
</tr>
<tr>
<td></td>
<td>5.33</td>
<td>7.11</td>
<td>4.23</td>
</tr>
<tr>
<td></td>
<td>5.55</td>
<td>4.60</td>
<td>5.73</td>
</tr>
<tr>
<td></td>
<td>5.72</td>
<td>4.23</td>
<td>4.50</td>
</tr>
</tbody>
</table>

mean 6.77 7.35 5.69 5.72 6.71 7.64 5.55 5.57 7.35 8.18 5.73 4.50
MATLAB SCRIPT FUMIGATION EXTRACTION METHOD (FE BIOMASS C PER GRAM OM)
FIEKE VLAAR - 10467165

CLEAR WORKING SPACE
close all
clear
clc

LOADING DATA
[P02 J02 M02 O02 P25 J25, M25 O25 P510 J510 M510 O510] = textread('FumigatieOmrekeningOM.txt','%f%f%f%f%f%f%f%f%f%f%f%f', 'delimiter ', 'tab', 'headerlines', 2);

ASSIGNING DATA TO VARIABLES
Pioneer02 = P02(1:6);
Pioneer25 = P25(1:6);
Pioneer510 = P510(1:5);
Jong02 = J02(1:6);
Jong25 = J25(1:6);
Jong510 = J510(1:5);
Middel02 = M02(1:6);
Middel25 = M25(1:6);
Middel510 = M510(1:6);
Oud02 = O02(1:5);
Oud25 = O25(1:6);
Oud510 = O510(1:6);

CHECKING DATA FOR OUTLIERS
boxplot(Pioneer02);
boxplot(Pioneer25);
boxplot(Pioneer510);
boxplot(Jong02);
boxplot(Jong25);
boxplot(Jong510);
boxplot(Middel02);
boxplot(Middel25);
boxplot(Middel510);
boxplot(Oud02);
boxplot(Oud25);
boxplot(Oud510);

CREATING GROUPS FOR CONDUCTING THE KRUSKALWALLIS TEST
Laag02 = [Pioneer02; Jong02; Middel02; Oud02];
Laag25 = [Pioneer25; Jong25; Middel25; Oud25];
Laag510 = [Pioneer510; Jong510; Middel510; Oud510];
Group1 = [ones(6,1); (ones(6,1)*2); (ones(6,1)*3); (ones(5,1)*4)];
Group2 = [ones(6,1); (ones(6,1)*2); (ones(6,1)*3); (ones(6,1)*4)];
Group3 = [ones(5,1); (ones(5,1)*2); (ones(6,1)*3); (ones(6,1)*4)];

CONDUCTING THE KRUSKALWALLIS TEST
[p, tbl, stats1] = kruskalwallis(Laag02, Group1);
[p, tbl, stats2] = kruskalwallis(Laag25, Group2);
[p, tbl, stats3] = kruskalwallis(Laag510, Group3);
multcompare(stats1)
multcompare(stats2)
multcompare(stats3)

multcompare(stats1)
multcompare(stats2)
multcompare(stats3)

%CALCULATING THE STANDARD DEVIATIONS%
std1= std(Pioneer02);
std2=std(Pioneer25);
std3=std(Pioneer510);
std4=std(Jong02);
std5=std(Jong25);
std6=std(Jong510);
std7=std(Middel02);
std8=std(Middel25);
std9=std(Middel510);
std10=std(Oud02);
std11=std(Oud25);
std12=std(Oud510);

%CREATING VARIABLES FOR BAR GRAPH WITH STANDARD DEVIATION%
std_fumigatie=[std1,std2,std3,std4,std5,std6,std7,std8,std9,std10,std11,std12];
y= [P02(7) P25(7) P510(7); J02(7) J25(7) J510(7); M02(7) M25(7) M510(7);
O02(7) O25(7) O510(7)];

%CREATING BAR GRAPH WITH STANDARD DEVIATION%
bb=bar(y'); hold all
data=repmat([1:4]'*ones(3,1)',1,1);
for i = 1:3;
    j = 1:4;
    x = -0.5 + i + 1/5 * j;
    errorbar(x, y(j,i), std_fumigatie(j,i), '.');
end

%COMPLETING THE BAR GRAPH WITH LABELS, TITLE AND LEGEND%
labels = {'0-2','2-5','5-10'};
set(gca, 'Xtick', 1:12, 'XtickLabel', labels);
title 'Microbial biomass content per gram OM based on the FE method'
ylabel 'FE biomass C (mg C/g OM)'
xlabel 'Soil depth (cm)'
ln={'Pioneer','Young','Middle','Old'};
legend(ln)

Correlation FE and Sir method per gram dry weight

%MATLAB SCRIPT CORRELATION FE AND SIR METHOD PER GRAM dry weight
%FIEKE VLAAR - 10467165

%CLEAR WORKING SPACE%
close all

clear
clc

%LOADING DATA%
[rP02 rJ02 rM02 rO02 rP25 rJ25 rM25 rO25 rP510 rJ510 rM510 rO510]=textread('RespicondOmrekeningVerschil.txt', '%f%f%f%f%f%f%f%f%f%', 'delimiter','tab','headerlines',2);
Correlation FE and SIR method per gram OM

%MATLAB SCRIPT CORRELATION FE AND SIR METHOD PER GRAM OM
%FIEKE VLAAR - 10467165

%CLEAR WORKING SPACE%
close all
clear
clc

%LOADING DATA%
[rP02 rJ02 rM02 rO02 rP25 rJ25 rM25 rO25 rP510 rJ510 rM510 rO510] =textread('RespicondOmrekeningOM.txt','%f%f%f%f%f%f%f%f%f%f%f%f', 'delimiter', 'tab','headerlines',2);

%CREATING X AND Y FOR CORRELATION%
Y = [rP02(7) rP25(7) rP510(7) rJ02(7) rJ25(7) rJ510(7) rM02(7) rM25(7) rM510(7) rO02(7) rO25(7) rO510(7)];
X = [rP02(7) rP25(7) rP510(7) rJ02(7) rJ25(7) rJ510(7) rM02(7) rM25(7) rM510(7) rO02(7) rO25(7) rO510(7)];

%CORRELATION%
[RHO, PVAL] = corr(X, Y)
plot(X, Y, 'r')
title('Correlation FE and SIR method per gram OM')
ylabel('FE method (mg C/g OM)')
xlabel('SIR method (µg C/h/g OM)')
### C: Moisture content per soil layer per heathland community – SIR method

Table 7: Equalized moisture content per soil layer per heathland community for the SIR method

<table>
<thead>
<tr>
<th>group</th>
<th>moisture content</th>
<th>group</th>
<th>Moisture content</th>
<th>Group</th>
<th>Moisture content</th>
</tr>
</thead>
<tbody>
<tr>
<td>P0-2</td>
<td>162.69</td>
<td>P2-5</td>
<td>37.15</td>
<td>P5-10</td>
<td>16.62</td>
</tr>
<tr>
<td>J0-2</td>
<td>242.36</td>
<td>J2-5</td>
<td>95.17</td>
<td>J5-10</td>
<td>17.53</td>
</tr>
<tr>
<td>M0-2</td>
<td>64.55</td>
<td>M2-5</td>
<td>36.33</td>
<td>M5-10</td>
<td>24.61</td>
</tr>
<tr>
<td>O0-2</td>
<td>187.22</td>
<td>O2-5</td>
<td>32.33</td>
<td>O5-10</td>
<td>16.39</td>
</tr>
</tbody>
</table>