



UMEÅ UNIVERSITY

Invasive Earthworms and their effect on Soil Organic Matter

Impact on Soil Carbon 'Quality' in Fennoscandian Tundra

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Bachelor thesis, 180 hp
Degree project in Earth Science, 15 hp
Spring term 2021

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Abstract

Arctic soils contain a large fraction of our planet's terrestrial carbon (C) pool. When tundra soils become warmer and permafrost thaws, non-native geoengineering earthworms can enter these soils and ingest organic matter accumulated over long timescales. Previous studies have found that earthworms increase mineralization rates of soil organic matter into carbon dioxide (CO₂) when introduced. Yet, this initial mineralization boost seems transient with time and it has been hypothesized that earthworms stimulate formation of persistent C forms. In this study, I investigated how non-native, geoengineering earthworms affected the relative proportions of seven carbon forms in the O and A₁ horizon of tundra soil and if their effect induced a change in pH. I used Nuclear Magnetic Resonance (NMR) spectroscopy to understand what happens to soil carbon compounds in two different tundra vegetation types (heath and meadow), that had been subjected to earthworm treatment for three summers. I found that O-aromatic C increased from 7.22% ± 0.24 (mean ± stderr) in the meadow soil lacking earthworms to 8.98% ± 0.30 in the meadow exposed to earthworms, and that aromatic C increased from 8.71% ± 0.23 to 9.93% ± 0.25. In similar, the result suggested that alkyl C decreased in this vegetation type from 20.43% ± 0.38 to 18.70% ± 0.25 due to earthworm activities. I found no effect on the chemical properties in the heath. I conclude that geoengineering earthworms affect the two vegetation types differently and that earthworms seem to enhance the accumulation of recalcitrant aromatic C forms.

Key words: Geoengineering earthworms, Nuclear Magnetic Resonance spectroscopy, carbon compound composition, ¹³C, Arctic tundra

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1 Introduction

In 2015, all member countries in the United Nations (UN) adopted seventeen sustainable development goals as an effort to work towards a more sustainable future, regarding both the planet and its people (Kroll, Warchold and Prajal 2019). One of these goals concerns life on land where a sub-goal is to reduce the impact of invasive alien species (IAS) and, to the extent possible, eradicate or control species with a high negative impact on biodiversity (United Nations 2015). Invasive alien species can be defined as an organism that has been accidentally or deliberately introduced, by human activities, into a habitat where it has a negative impact (Brunel et al. 2013). When discussing IAS, one usually visualizes plants or larger fauna which are easier to conceptualize as a threat to the environment, while smaller creatures moving around in the soil itself are rarely considered (Bohlen et al. 2004; Early et al. 2016). However, in North America for instance, earthworms were eradicated during the last ice age but have, over the last centuries, repopulated the formerly glaciated forests due to various human activities, such as fishing and farming (Bohlen et al. 2004). The repopulation has caused a drastic disturbance in soils and the non-native earthworms are therefore considered as IAS (Resner et al. 2015).

In Fennoscandia, earthworms are so far not considered as invasive, as they are in North America, but the establishment of some earthworm species in sub-arctic environments of northern Sweden has been argued to be driven by human introductions (Wackett et al. 2018). More specifically, the study by Wackett et al. (2018) argued that sub-arctic areas are currently mainly inhabited by earthworms that have surface litter as their main habitat (epigenic earthworms), while humans introduce endogeic and anecic species that dig deeper into the mineral soil and produce both vertical and horizontal burrows (Brown, Barois and Lavelle 2000). It is through these burrows, together with castings and middens, that these latter two earthworm groups can cause substantial transformation and reallocation of soil nutrients and thus, are often referred to as 'geoengineering earthworms' or 'ecosystem engineers' (Brown, Barois and Lavelle 2000). An establishment of geoengineering earthworms in the Fennoscandian arctic may be problematic because of their ability to restructure nutrient pools and reshape morphology in the soil horizons, which can have consequences that are not yet fully understood (Blume-Werry et al. 2020).

Soils in the Arctic have been calculated to contain approximately 1672 Pg, (i.e., 1672 billion tons) of organic carbon (C), which is two times more than the current atmospheric C content (Tarnocai et al. 2009). If only portions of this C are degraded and released as methane (CH₄) or carbon dioxide (CO₂), there is potential, and large concerns, for a massive positive feedback (Schuur et al. 2015). However, the rate at which this can occur is partly controlled by the decomposition of soil organic C (Schuur et al. 2015). Lubbers et al. (2013) found an increase in soil CO₂ emissions by 33% with the presence of earthworms, which is thought to be caused by their geoengineering capacity. However, the same study found no significant effect on the soil C pool and that the effect on CO₂ emissions decreased with time and ceased to be significant after 200 days. This indicates that earthworms may change the C compound composition in the soil and might, at least initially (<200 days), reshape it into a more readily decomposable form. Because soils in ecosystems dominated by a cold climate seem to be in the early stages of decomposition, increasing temperatures creates a risk of rapid soil organic carbon (SOC) loss (Semenchuk et al. 2019). Carbon compounds that are more readily degradable because of their composition may be especially at risk in a warmer climate (Semenchuk et al. 2019). The presence of geoengineering earthworms could potentially enhance the degradation if they produce a more readily decomposable C form. What we do not know is whether earthworms change the soil C compound composition and thereby quality, in the Fennoscandian tundra. Can the geoengineering earthworm actually change the chemical property of soil organic matter (SOM)?

Depending on the molecular structure, different forms of SOM degrade easier than others (Bosatta and Ågren 1999). Soil organic matter that is more easily accessible for microbial

degradation and plant uptake is considered to contain high-quality C compounds and according to the enzyme-kinetic hypothesis, this is the form of C that takes the least amount of activation energy to degrade (Bosatta and Ågren 1999). The opposite is hypothesized for low-quality C compounds (also called recalcitrant molecular structures), i.e., organic compounds that requires higher activation energy to degrade (Bosatta and Ågren 1999). However, the decomposition process of SOM is complex and varies depending on different factors, e.g., heterotrophic microorganisms and the enzymes they excrete, the stage of substrate decay, sorption on- and physical protection through minerals (Wagai et al. 2013). Soil organic matter also has the ability to change soil pH depending on the chemical functional group, the rate at which litter is added to the soil, decomposition rate, and the initial soil pH (Rukshana et al. 2011).

Traditionally, SOM has been studied mainly through a technique called alkaline extraction, which has been used in different forms for over 200 years to separate the mineral phase from the organic phase (Lehmann and Kleber 2015). The main principle of this technique in modern times uses a solution of sodium hydroxide (pH 13) to ionize oxygen-containing functional groups, forcing them into a more soluble state (Lehmann and Kleber 2015). The organic material is reacidified so that humic acids precipitate (Lehmann and Kleber 2015). After reacidification, the organic matter that is still soluble is called fulvic acid (Lehmann and Kleber 2015). The problem with this technique is that 50-70% of the studied matter is left unextracted (called humin), which makes it an inexact and non-trustworthy method (Lehmann and Kleber 2015).

Today, SOM can be studied with much more exact methods, one of them being Nuclear Magnetic Resonance (NMR) spectroscopy, where the samples can be analysed without disturbing the molecular structure (Mlynárik 2017; Schmidt et al. 2011). In short, the sample (which in the case of ^{13}C has a nuclear spin of $\pm 1/2$ meaning that it has a magnetic moment) is placed in the centre of a strong magnetic field which forces the nuclei to orientate along the Z-axis (Mlynárik 2017). A radiofrequency pulse is applied which disturbs the nuclei so that they tilt towards the XY plane, then, as the nuclei re-aligns with the Z-axis, they induce a decaying current called free induction decay (FID) (Mlynárik 2017). In a molecule, each nucleus is exposed to a different magnetic field depending on the electron density surrounding it, which makes the nuclei react and recover differently to the applied radiofrequency pulse, creating a unique FID (Mlynárik 2017). Depending on how the ^{13}C nuclei behave in this process, we can tell how they are arranged in the molecule and thus, differentiate different molecules (Mlynárik 2017).

My bachelors' thesis aim to increase our understanding regarding how geoengineering earthworms may change the SOM quality of arctic soils. The purpose of this study is to investigate if and/or how earthworms alter common C-based functional groups found within tundra soils developed under two different tundra vegetation types: heath and meadow. My main hypotheses were:

- 1) The chemical composition of SOM in the O and A₁ horizon of tundra soils is altered by earthworms.
- 2) There is a difference in earthworm effect on carbon compound composition between the two vegetation types: heath and meadow.
- 3) Earthworms, directly or indirectly, affect carbon quality in the O and A₁ horizon to the degree that pH is altered.

My three hypotheses were tested through NMR analysis and pH measurements of topsoil (O/A₁ horizons) from mesocosms, which are outdoor and partially enclosed experimental plots,

established in 2013 and subjected to treatments with geoenvironmental earthworms between 2017 and 2020.

2 Method and materials

2.1 Study site description

The outdoor experiment was performed at Abisko Scientific Research Station in the Fennoscandian arctic (Fig. 1) by Blume-Werry et al. (2020). The mean annual temperature is approximately 0°C while the mean annual precipitation is approximately 335 mm (Blume-Werry et al 2020). The research station lies 380 meters above sea level within a zone of sporadic permafrost (Blume-Werry et al 2020). Nuclear Magnetic Resonance (NMR) analysis was performed at the Chemical Institute at Umeå University.

2.2 Experimental design

Complete details are found in Blume-Werry et al (2020). In short, 48 mesocosms were prepared with dwarf shrub-dominated heath in 24 of them and forb-dominated meadow in the remaining 24. Geoenvironmental earthworm species *Lumbricus Rubellus* (epi-endogenic), *Lumbricus Terrestris* (anecic), and *Aporrectodea* sp (endogenic) were added to 12 mesocosms of each vegetation type while the remaining 24 mesocosms were kept worm-free as controls. The monoliths were kept undisturbed for approximately 5 years before the worm treatment was added. The treatment ran for 3 summers before samples were collected and analysed.

2.3 Soil preparation and chemical analysis

I added 20ml H₂O to 2g of dried soil and shook the samples for 24h whereafter pH was measured. Thereafter, 10ml CaCl₂ was added to the samples and shook again for 30 seconds, pH was measured a second time 5 minutes after shaking.

Dried soil from each top organic layer (O and A1 horizons) was milled using a ball mill as preparation for the NMR spectroscopy. The samples were not washed nor treated in any other way. Each 4 mm ZrO₂ rotor and KEL-F cap was weighed before a dried and milled soil sample from the organic horizon (O/A1) in each mesocosm were compacted into the rotor, sealed with the KEL-F cap, and weighed again. This resulted in approximately 100 mg of soil for each sample.

Experiments were performed with ¹³C Cross-Polarization Magic Angle Spinning (CP-MAS) on a Bruker 500 MHz Avance III spectrometer. The CP-MAS spectra were acquired at a spin rate of 10kHz, using a 1.5ms contact time with ¹³C at 60kHz and a 1H ramp of 45-90kHz to sweep both +1 and -1 sidebands and achieve stable polarization transfer. SPINAL64 1H decoupling at 83kHz was applied during acquisition and followed by a 2s relaxation delay. The number of scans, and therefore experimental time, differed depending on previously calculated loss on ignition (LOI); 4000 scans (LOI 60-89%), 8000 scans (LOI 40-59%), and 16000 scans (LOI < 40%). The obtained data were processed in the Topspin (version 3.6.2) software.

2.4 Processing of raw NMR spectra/separation into integrals

Each spectrum was integrated, by Tobias Sparrman, into chemical shift regions according to Smernik (2005): 0–50 ppm (alkyl C), 50–60 ppm (methoxy/N-alkyl C), 60–93 ppm (O-alkyl

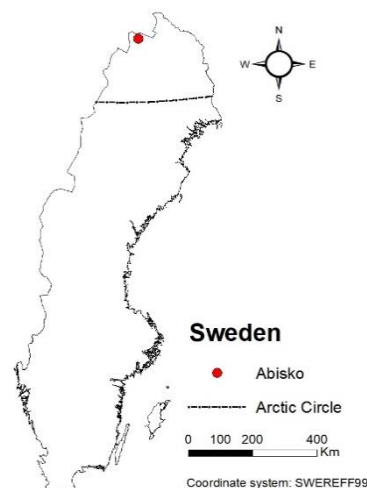


Figure 1. Overview map showing the location of Abisko (red dot) and the arctic circle (dotted black line). Coordinate system : SWEREF99_TM. Data source: Länsstyrelsen (2021).

C), 93–112 ppm (di-O-alkyl C), 112–140 ppm (aromatic C), 140–165 ppm (O-aromatic C), and 165–190 ppm (carbonyl C). Thereafter, the spectrum from each mesocosm was adjusted into norm relative form, i.e., the sum of integral 1-7 equalled 100 for each mesocosm.

2.5 Statistical analysis

Statistical analyses were carried out using the SPSS (version 3.6.3) software. My first two research questions were tested with two-way ANOVA:s with each of the 7 norm relative integrals as the dependent variable and vegetation type (heath and meadow) and treatment (worms and no worms) as fixed variables. My third research question was also similarly tested with a two-way ANOVA, with vegetation type and treatment as fixed variables and pH (H₂O and CaCl₂ respectively) as the dependent variable. Multidimensional scaling (PROXSCAL) was also performed in SPSS with vegetation type, treatment, and all 7 integrated carbon compound relative proportions chosen as variables. The confidence interval was set to 95% for all statistical analyses. To visualize where differences occur in the ANOVA:s, bar charts were created for each of the 7 integrals with standard error chosen as error bars. The same structure was followed for pH plots. Coordinates obtained from multidimensional scaling were plotted in an XY-scatter plot.

3 Results

The chemical shifts were calibrated externally to the adamantane CH₂-signal at 38.48 ppm, after 10 days the drift was 0.08ppm which is negligible for this study. The signal to noise (S/N) ratio for the reference sample was 125 at the O-alkyl C top (Fig. 2), the lowest S/N ratio obtained was 30 whereas the majority of samples had an S/N ratio between 50 and 100 which is acceptable for this study.

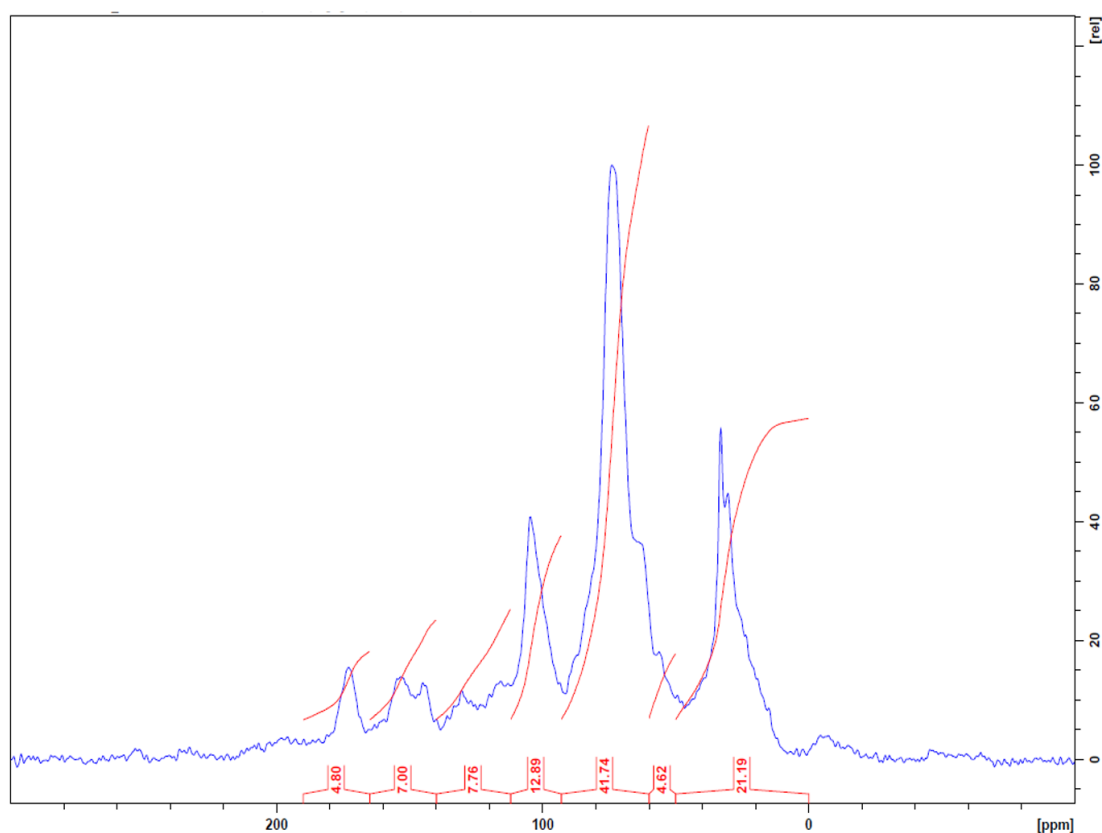


Figure 2. Signal to noise (S/N) ratio for the reference sample. Shift regions are read from right to left on the X-axis: 0–50 ppm (alkyl C), 50–60 ppm (methoxy/N-alkyl C), 60–93 ppm (O-alkyl C), 93–112 ppm (di-O-alkyl C), 112–140 ppm (aromatic C), 140–165 ppm (O-aromatic C), and 165–190 ppm (carbonyl C).

3.1 pH measurements

$\text{pH}_{\text{H}_2\text{O}}$ in the heath and meadow was 4.53 ± 0.13 (mean \pm stderr) and 5.31 ± 0.14 , respectively. For $\text{pH}_{\text{H}_2\text{O}}$ there were no treatment or interaction effect, but there was a significantly lower pH in the heath (vegetation, $F_{1,48} = 34.286$, $P = 0.000$) (Fig. 3ab). $\text{pH}_{\text{CaCl}_2}$ in the heath and meadow was 4.22 ± 0.17 and 4.93 ± 0.10 , respectively. $\text{pH}_{\text{CaCl}_2}$ measurements were also significantly lower in the heath (vegetation, $F_{1,48} = 39.948$, $P = 0.000$), but there were no interaction effect nor treatment effects (treatment, $F_{1,48} = 3.182$, $P = 0.081$). Regardless of chemical treatment, pH was significantly lower in the heath than in the meadow.

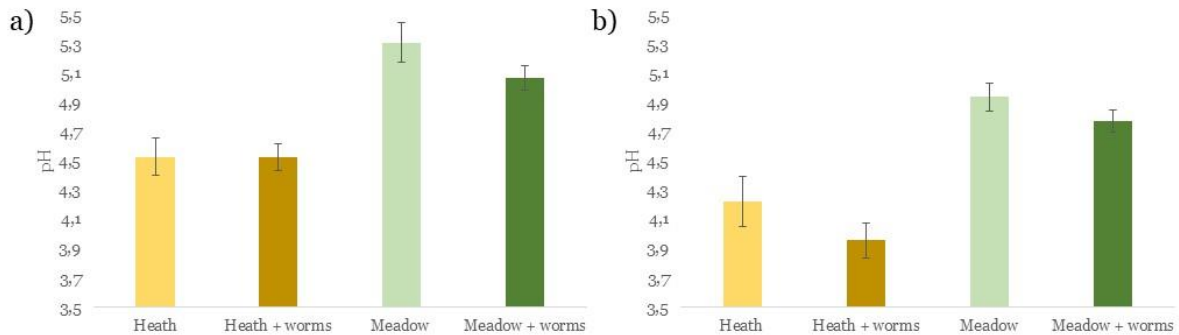


Figure 3. Mean pH measurements for soil samples in a) H_2O , and b) CaCl_2 . Error bars represent standard error.

3.2 Nuclear Magnetic Resonance

The results of the NMR analysis are shown in figures 4-7. An interaction effect between vegetation type and treatment increased the relative proportions of O-aromatic C from $7.22\% \pm 0.24$ (mean \pm stderr) to $8.98\% \pm 0.30$ (vegetation \times treatment, $F_{1,48} = 14.312$, $P = 0.000$) and aromatic C from $8.71\% \pm 0.23$ to $9.93\% \pm 0.25$ (vegetation \times treatment, $F_{1,48} = 8.024$, $P = 0.007$). Furthermore, the interaction effect decreased the relative proportions of alkyl C from $20.43\% \pm 0.38$ to $18.70\% \pm 0.25$ (vegetation \times treatment, $F_{1,48} = 6.265$, $P = 0.016$). This effect is explained by worm treatment and meadow vegetation (Fig 4a-c). Treatment alone shows a significant increase in O-aromatic C (treatment, $F_{1,48} = 7.170$, $P = 0.010$) and aromatic C (treatment, $F_{1,48} = 5.237$, $P = 0.027$), but not for alkyl C. Relative proportions significantly differs between the heath and the meadow for all the aforementioned carbon compounds; O-aromatic C (vegetation, $F_{1,48} = 5.919$, $P = 0.019$) and aromatic C (vegetation, $F_{1,48} = 25.976$, $P = 0.000$) had larger proportions in the meadow than in the heath whereas alkyl C (vegetation, $F_{1,48} = 46.880$, $P = 0.000$) had larger proportions in the heath than in the meadow. Looking at the interaction effect and bar charts, these three carbon compounds are significantly affected in meadow but not in heath.

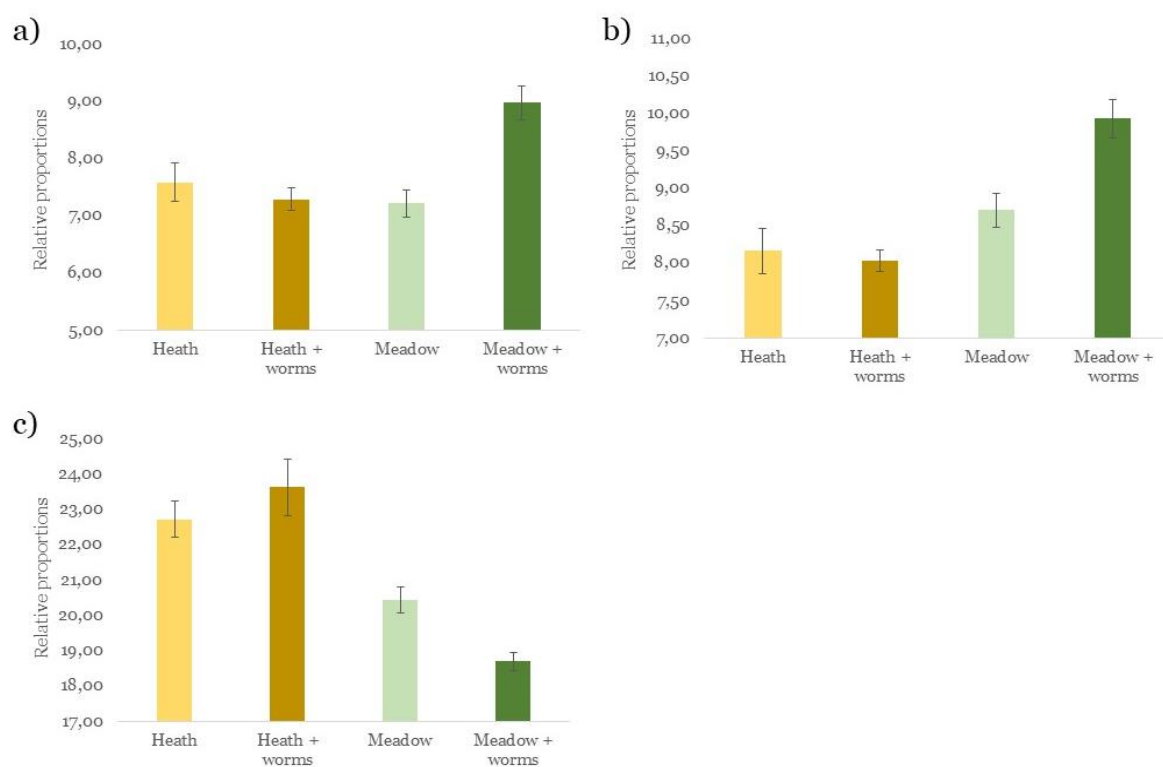


Figure 4. Relative proportions (Y-axis) of a) O-aromatic C, b) aromatic C, and c) alkyl C in heath and meadow with and without worm treatment. Error bars represent standard error. The Y-axis differ between C compounds to better visualize differences between treatments and vegetation.

Carbonyl C, di-O-alkyl C and methoxy/N-alkyl C showed no significant effect in relative proportions regarding neither worm treatment nor interaction effect (Fig. 5a-c). There were, however, a significant difference regarding relative proportions of the C forms between the two vegetation types, where carbonyl C (vegetation, $F_{1,48}=86.384$, $P=0.000$) and methoxy/N-alkyl C (vegetation, $F_{1,48}=62.430$, $P=0.000$) had larger relative proportions in the meadow whereas di-O-alkyl C (vegetation, $F_{1,48}=4.953$, $P=0.031$) had a larger relative proportion in the heath.

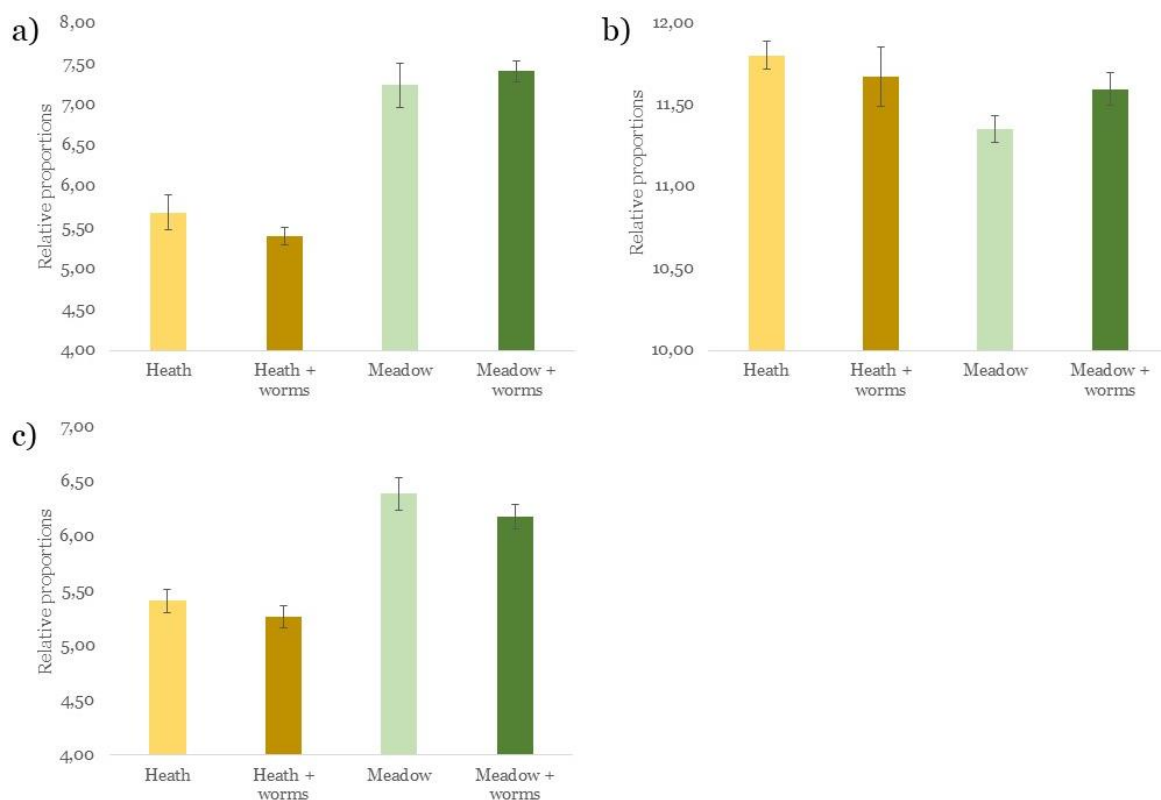


Figure 5. Relative proportions (Y-axis) of a) carbonyl C, b) di-O-alkyl C, and c) methoxy/N-alkyl C in heath and meadow with and without worm treatment. Error bars represent standard error. The Y-axis differ between C compounds to better visualize differences between treatments and vegetation.

No significant effect of the two variables alone or interaction effect was obtained regarding O-alkyl C, which is a labile form of C. The box plot indicate a decrease in mean O-alkyl C in the meadow with treatment but the error bar show a large variation in the data (Fig. 6).

The multidimensional XY-scatter plot show that when combining all carbon compounds, the two vegetation types differ in large, but also that the main difference regarding treatment lies within meadow vegetation, where overlapping between samples is low (Fig. 7).

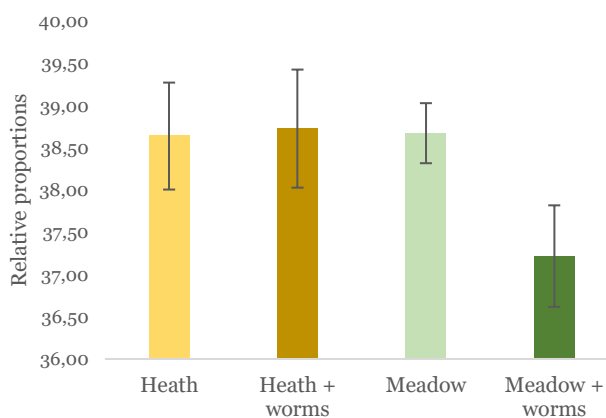


Figure 6. Relative proportions (Y-axis) of O-alkyl C in heath and meadow with and without worm treatment. Error bars represent standard error.

No matter treatment or vegetation type, the relative proportion of O-alkyl C was the largest with mean values between 37.2 % (meadow with treatment) and 38.7 % (heath with treatment) (Fig. 6). Second largest relative proportion, again no matter treatment or vegetation type, was alkyl C with mean values between 18.7% (meadow with treatment) and 23.6% (heath with treatment) (Fig. 4c). Remaining carbon compounds (O-aromatic C, aromatic C, carbonyl C, di-O-alkyl C and methoxy/N-alkyl C) show overlapping relative proportions depending on treatment and vegetation type (Fig. 4ab; Fig. 5ac).

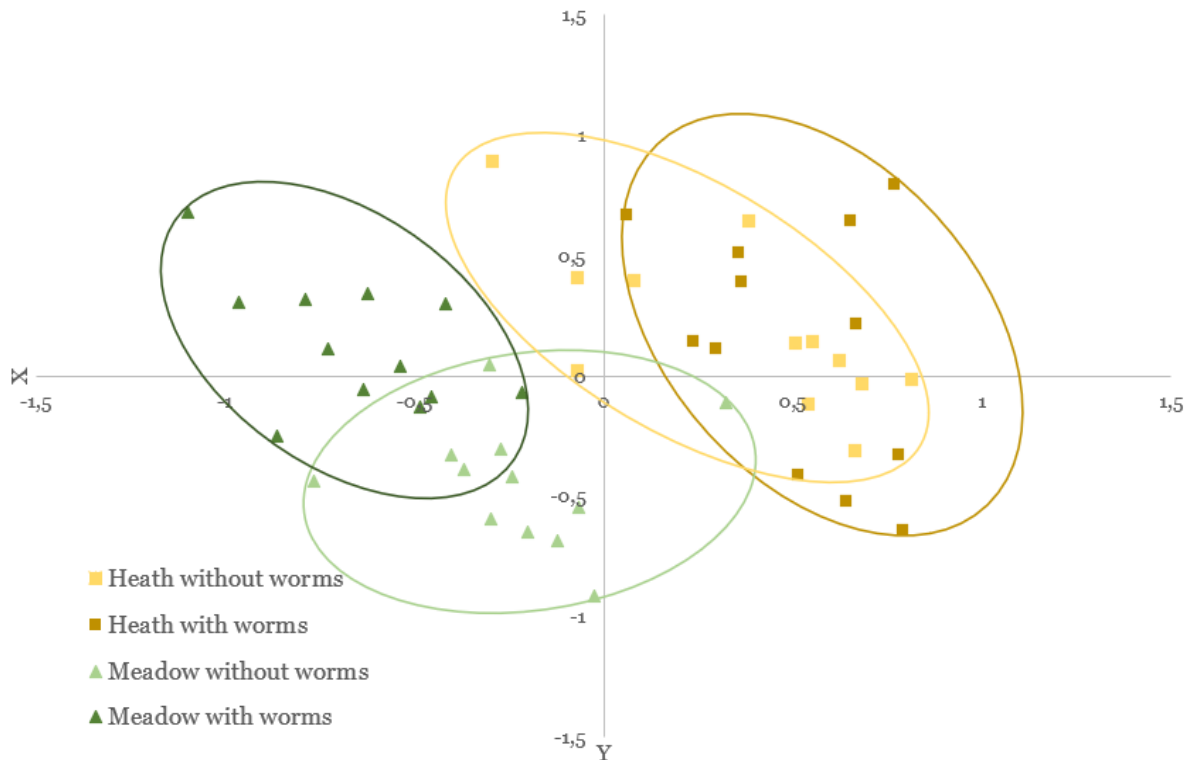


Figure 7. Multidimensional scaling of the variables heath and meadow with and without worm treatment, with calculated coordinates plotted in an XY scatter plot. Circles encompass all samples for each category.

4 Discussion

4.1 Earthworm impact on the chemistry of SOM

In my first hypothesis, I stated that the chemical composition of SOM in the O and A₁ horizon of tundra soils are altered by earthworms. My results show that the relative proportions of O-aromatic C and aromatic C increased whereas alkyl C decreased in the treated meadow (Fig. 4). Hence, my findings support hypothesis 1. Because aromatic compounds are considered recalcitrant (originating from lignin and tannins), it is no surprise that earthworms increased the relative proportions of O-aromatic and aromatic C (Kögel-Knabner 2002). What is interesting, however, is that alkyl C (originating from lipids, e.g., cutin and surface waxes) has decreased in the meadow but not in the heath because this C compound is also considered recalcitrant (Erhagen et al. 2013; Kögel-Knabner 2002). Normally, O-alkyl C and di-O-alkyl C (originating from carbohydrates) decrease whereas alkyl C increase with degradation according to previous warming experiments (Erhagen et al. 2013; Semenchuk et al. 2019). Furthermore, no significant increase or decrease is seen in carbonyl C (a component of lipids or amino acids), O-alkyl C, di-O-alkyl C, and methoxy/N-alkyl C (originating from lignin and proteins) (Fig. 5; Fig. 6) (Erhagen et al. 2013; Kögel-Knabner 2002). As mentioned, C compounds in lipids and lignin are mainly considered recalcitrant and I, therefore, conclude that the results regarding carbonyl C and methoxy/N-alkyl C are as expected. However, O-alkyl C and di-O-alkyl C are carbohydrates which are considered easily degradable in warming experiments (Erhagen et al. 2013; Semenchuk et al. 2019). Therefore, it seems like the effect of geoen지니어링 earthworms on carbon forms differs from the warming effect on carbon compounds, with the exception of aromatic C which increases in both cases, as illustrated in figure 8.

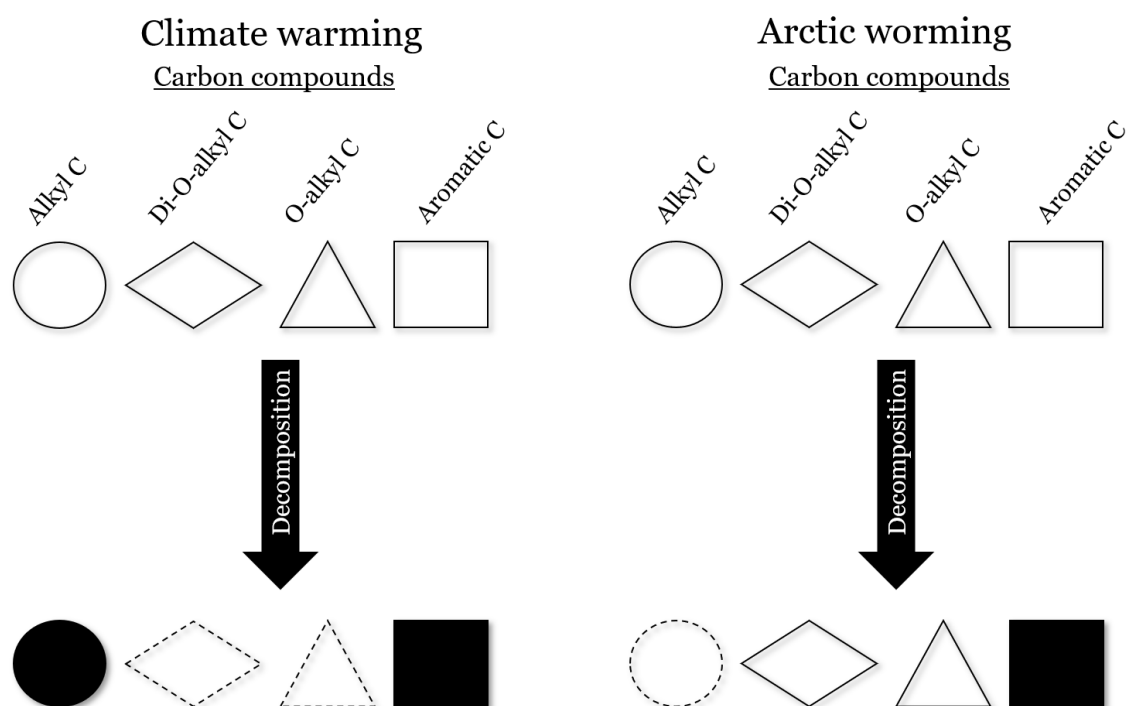


Figure 8. Conceptual figure of the difference between degradation of C compounds due to climate warming and arctic warming (i.e. the earthworm effect). Filled shapes represent an increase, shapes with dotted outlines represent a decrease and shapes with solid lines represent no change. Sources: Erhagen et al. 2013 and Semenchuk et al. 2019.

In my second hypothesis, I stated that there is a difference in earthworm effect on C compound composition between the two vegetation types: heath and meadow. A significant effect of worm treatment was seen in the meadow (Fig. 4); hence, my findings also support hypothesis 2, geoengineering earthworms have a larger effect in meadow vegetation. Blume-Werry et al. (2020) found that geoengineering earthworms only reduced the surface litter in the meadow, meaning that they pull the nutrients from the surface litter down in the meadow soil but not in the heath. It therefore seems like geoengineering earthworms have different areas of feeding in the two vegetation types, where they feed on fresh surface litter in the meadow and on mineral soil or humic substances in deeper soil horizons in the heath. This could explain the lack of effect that is seen in heath in this study. In other words, I cannot exclude that an effect on chemical compound composition exists in deeper horizons also in the heath. The difference in effect between vegetation types might depend on several different factors, one factor being that the two vegetation types are inhabited by plant species with different chemical compound composition and thus are affected unequally (Kögel-Knabner 2002). This is demonstrated by the fact that relative proportions significantly differed between vegetation types regarding all C forms except O-alkyl C, regardless of treatment, or interaction effect. However, there is no clear pattern in my results explaining why this difference in effect occurs. Currently, I cannot fully resolve the explanations behind my results; however, the following four paragraphs list possible mechanisms in which geoengineering earthworms may have altered the C-forms in my samples.

Theory 1: the changing plant community effect. Blume-Werry et al. (2020) found that earthworms increased the nitrogen (N) concentrations in plants in the Fennoscandian tundra, but that the N increase affected plant species differently. Some plants increased their biomass by growing taller or producing longer or quantitatively more floral shoots while other species were not affected. Vegetation types contain different C compositions and because only some plants increase in biomass, the chemical composition of those plants will increase in the soil

(Preston and Trofymow 2000). Thus, my first theory is *that geoengineering earthworms might change the vegetation type into one that, in this case, contains less lipids and waxes, which is the reason for the decrease in relative proportions of alkyl C in the meadow.*

Theory 2: the preferential degradation of alkyl C. The warming studies have shown that alkyl C is recalcitrant when it comes to degradation due to increasing temperature (Erhagen et al. 2013; Semenchuk et al. 2019), but might it be that the earthworm gut (with metabolic processes and microbes) can decompose this carbon form? Previous studies have also shown that earthworms cannot degrade lignin, i.e. aromatic structures, without the help of microorganisms (more specifically fungi) (Bossuyt et al. 2001; Curry and Schmidt 2007). This leads to my second theory, *that alkyl C is degraded while the aromatic structures are not because alkyl C originates from lipids and waxes, not lignin. Therefore, alkyl C might be directly available to geoengineering earthworms while aromatic structures are not.*

Theory 3: the preference for lipid-containing food sources. My third theory is *that the anecic species in this study (*L. Terrestris*) favour lipid-containing matter as a food source and have simply reallocated the alkyl C from the O/A₁ horizon to the rooting zone. In contrast, they have deselected the aromatic structures because of the limitation in lignin degradation.* Reports on *L. Terrestris* feeding preferences differ and no literature is found to support the theory that *L. Terrestris* prefer alkyl C as a food source. However, Knollenberg, Merritt and Lawson (1985) observed this earthworm species effectiveness in consuming fresh leaf litter (containing alkyl C in the form of surface waxes), pulling it into their deep burrows. It is also commonly known that anecic species are surface feeders who burrow deep in the ground (Hale et al. 2005).

Theory 4: the microorganism decrease effect. According to Kögel-Knabner (2002), both plants and microorganisms contain lipids (thus, alkyl C). The study by Blume-Werry et al. (2020) found that geoengineering earthworms significantly decrease the number of microorganisms in the meadow but not in the heath. Thus, my fourth theory is *that the decrease seen in alkyl C in the meadow might partly depend on earthworm altering microbial biomasses.*

4.2 pH measurements in relation to carbon compounds

In my third hypothesis, I stated that earthworms, directly or indirectly, affect carbon quality in the O and A₁ horizon to the degree that pH is altered. I did not find any significant results regarding treatment or interaction effect on this matter, although there are indications that pH_{CaCl₂} is decreased in the presence of earthworms (P=0.081). Ferilian et al. (2020) found that earthworms generally increase soil pH due to decreases in organic layers and upward transport of soil with a higher pH. On the other hand, Rukshana et al. (2011) found that the effect of SOM composition on pH is partly dependent on, among other parameters, initial soil pH and decomposition rate. The decomposition rate is low in the arctic, which could be a potential explanation for the lack of change in my soil samples (Schmidt et al. 2011; Semenchuk et al. 2019). Measurements in pH_{H₂O} and pH_{CaCl₂} differ, but significant results were obtained regarding the difference between vegetation types in both chemical treatments. The heath shows lower pH values than the meadow, this result is supported by Björk et al. (2007) who reported higher pH values in the meadow than in the heath at Latnjajaure Field Station (15km west of Abisko). If the lower pH in the heath could be a possible explanation for the lack of earthworm effect in this vegetation is unclear, but it needs to be taken into consideration in future work.

4.3 Conclusions and further research

The results of this study seem to depend upon several factors such as microbial biomass and litter composition but the main factor initiating this change in C compound composition is geoengineering earthworms. There is no indication of earthworm induced change towards more readily decomposable C compounds because the more temperature labile forms are not

significantly affected in this 3-year perspective. However, it is clear that non-native earthworm introduction in the sub-arctic areas affects vegetation types differently and that alkyl C, which is recalcitrant to temperature, is not as recalcitrant or persistent in the presence of earthworms. Therefore, to fully understand what will happen to the sub-arctic carbon pools, one should study the interaction effect of earthworms and temperature. Moreover, my results further support Lubbers et al. (2013) in that earthworms seem to enhance the initial degradation of SOM, but that the accumulation of recalcitrant aromatic C forms might reduce degradation in the long term without affecting the total carbon pool.

Nuclear Magnetic Resonance proved to be a useful tool for this study. My recommendation for future research is to include a depth analysis to be able to understand if the carbon forms are reallocated to other soil horizons, but also to include absolute values and not only relative proportions. The aforementioned approach would clarify just how much the different C compounds have actually increased or decreased.

5 Acknowledgements

First of all, I would like to thank Jonatan Klaminder for all the time you spent answering my questions and guiding me through this process. As my supervisor, you have been a great support during my bachelors' thesis regarding everything from scientific writing to self-confidence, and a much-appreciated sounding board when things felt confusing. Secondly, I would like to thank Tobias Sparrman for the assistance and guidance regarding the NMR analysis and for making my time in the lab enjoyable. Finally, I would like to aim a warm thank you to all my teachers and fellow students at EMG for three fantastic years of learning.

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Appendix

Appendix 1. Norm relative data

The seven carbon compounds in norm relative form for each mesocosm, together with horizon, vegetation type, treatment, loss on ignition and sample weight.

SampleID	Horizon	Vegetation type	Treatment	LOI (%)	Weight sample (g)	Carbonyl C	O-aromatic C	Aromatic C	Di-O-alkyl C	O-alkyl C	Methoxy/N-alkyl C	Alkyl C	
1	A1	meadow	Worms	35,0	0,120	7,96	10,34	10,80	11,86	34,89		5,92	18
2	A1	meadow	Worms	29,5	0,130	8,17	11,20	11,60	11,28	32,71		5,84	19
3	O	heath	Worms	87,1	0,098	5,69	6,96	7,80	12,55	42,23		4,97	20
4	O	heath	Worms	89,4	0,095	4,80	7,00	7,76	12,89	41,74		4,62	21
5	O	meadow	Worms	42,8	0,122	7,14	8,57	9,07	12,02	39,72		5,78	18
6	O	heath	Worms	76,5	0,105	5,81	8,35	9,26	11,54	35,09		4,95	25
7	O	heath	Worms	62,5	0,108	5,98	7,48	8,49	12,19	40,16		4,90	21
8	O	heath	No Worms	84,5	0,102	5,73	7,81	8,82	12,47	39,93		4,80	20
9	O	meadow	No Worms	45,3	0,114	8,95	8,06	10,13	11,25	35,69		6,43	19
10	O	meadow	No Worms	63,5	0,109	7,91	7,37	9,25	11,68	38,70		6,28	19
11	O	meadow	No Worms	65,4	0,104	7,12	6,43	8,33	11,33	39,72		6,98	20
12	O	heath	No Worms	70,7	0,101	5,59	7,18	7,77	11,95	38,91		5,22	23
13	O	heath	Worms	77,8	0,110	5,59	8,01	7,92	11,95	40,51		5,27	21
14	O	heath	Worms	61,9	0,117	5,32	7,92	8,29	11,42	38,26		5,58	23
15	O	heath	Worms	55,3	0,111	5,66	7,88	8,27	11,58	37,99		5,70	23
16	A1	meadow	Worms	31,6	0,131	7,35	7,90	9,04	11,59	38,72		6,59	19
17	A1	meadow	Worms	39,5	0,127	6,48	7,66	8,54	11,72	40,36		6,22	19
18	O	meadow	No Worms	75,5	0,104	7,48	5,45	7,47	10,86	40,82		6,68	21
19	O	meadow	No Worms	45,8	0,108	7,37	6,99	8,72	11,12	39,14		6,85	20
20	O	heath	No Worms	73,6	0,098	5,03	6,95	7,64	11,77	41,09		5,46	22
21	O	meadow	No Worms	65,1	0,106	6,79	6,85	7,81	11,40	39,39		6,71	21
22	O	heath	No Worms	51,4	0,117	6,14	8,82	8,61	11,63	36,12		5,30	23
23	O	meadow	No Worms	59,9	0,112	7,41	7,71	9,16	11,44	37,88		6,61	20
24	O	heath	No Worms	84,4	0,096	5,10	7,14	8,03	11,94	38,80		5,20	24
25	A1	meadow	Worms	29,1	0,132	7,32	8,90	10,22	10,87	35,79		6,87	20
26	A1	meadow	Worms	47,8	0,115	7,76	9,54	10,63	11,98	36,86		5,91	17
27	A1	heath	Worms	73,0	0,104	5,23	7,40	8,07	11,18	36,39		5,70	26
28	A1	meadow	Worms	38,7	0,129	6,96	8,09	9,23	11,48	38,30		6,51	19
29	A1	heath	Worms	83,4	0,097	4,84	6,82	8,08	10,82	35,91		5,23	28
30	A1	meadow	Worms	51,6	0,115	7,49	9,28	10,19	11,91	37,59		5,60	18
31	A1	heath	Worms	74,8	0,105	5,45	6,93	7,45	11,06	36,45		5,59	27
32	O	heath	No Worms	62,0	0,114	6,71	8,88	9,09	11,76	36,31		5,48	22
33	O	heath	No Worms	69,0	0,108	4,95	6,41	6,99	11,34	39,17		6,03	25
34	O	heath	No Worms	29,1	0,133	6,08	10,20	10,25	11,98	33,85		5,05	23
35	O	meadow	No Worms	55,8	0,109	7,41	7,80	9,55	11,79	38,41		5,59	19
36	A1 / O	meadow	No Worms	66,8	0,106	7,13	6,55	8,15	10,93	39,01		6,72	22
37	O/A	heath	Worms	63,4	0,110	5,05	6,77	7,39	11,74	40,99		5,42	23
38	A1	meadow	Worms	39,9	0,126	7,49	8,38	9,85	11,52	37,45		6,58	19
39	A1	meadow	Worms	37,6	0,129	7,43	9,21	10,42	11,51	37,10		6,23	18
40	A1	heath	Worms	85,5	0,095	5,29	5,88	7,64	11,13	38,98		5,25	26
41	A1	meadow	Worms	35,7	0,129	7,34	8,65	9,56	11,38	37,10		6,10	20
42	O	meadow	No Worms	64,1	0,109	7,36	7,75	8,85	11,48	38,87		6,20	19
43	O	heath	No Worms	80,6	0,099	4,88	6,64	7,32	11,74	39,92		5,52	24
44	O	meadow	No Worms	82,6	0,105	4,78	8,40	8,00	11,65	38,38		5,17	24
45	O	heath	No Worms	62,3	0,110	7,22	7,58	9,18	11,84	39,44		6,06	19
46	O	heath	No Worms	70,7	0,110	5,46	7,13	7,67	11,40	38,66		5,25	24
47	O	heath	No Worms	73,9	0,103	5,27	6,30	6,61	11,76	41,43		5,55	23
48	O/A	meadow	No Worms	62,1	0,108	7,11	7,24	9,05	11,27	38,04		6,44	21