University of Zurich[UZH]

Root Litter Decomposition and Associated Carbon Fluxes in Degraded Peat Soils

GEO 511 Master's Thesis

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Abstract

Peatlands are one of the most critical global carbon (C) sinks, but they can become a C source when drained for human uses such as agriculture, and consequently affect our climate. One key unknown in peatland C cycling is the contribution of root-derived C to C fluxes and stocks, and how these dynamics change with peatland drainage. My study investigated the response of the C fluxes and stocks to five different ^{13}C labelled root litter amounts (+2.3 mg to 9.3 mg additional roots) and two different soil moistures (field moist, representing drained conditions, and saturated). As the amount of roots increased, respiration of CO_2 and CH_4 increased, but more carbon was also stored in the soil. Proportionally more of the newly added roots were respired than native peat carbon. The added root litter also triggered a priming effect for both CO_2 and CH_4 emitted from native peat, but the CH_4-C priming effect was up to 20 times higher than the CO_2-C priming effect. Nevertheless, despite the priming effect, slightly more of the root C remained in the soil than what was respired, suggesting that increasing root inputs to peat could potentially increase C sequestration. Overall, my study elucidates the strong and interactive effects of root litter and moisture on peatland C fluxes and storage and provides a first look into how processes such as priming can influence peatland C cycling.

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Abbreviations

C	Carbon
CH_4	Methane
C_{native}	Native-derived carbon
CO_2	Carbon dioxide
C_{root}	Root-derived carbon
C_{total}	Total carbon (= C_{native} + C_{root})
EC	Electrical conductivity
f_{root}	Proportion of root-derived C
M	Molar mass
$NaCO_3$	Sodium carbonate
NaOH	Sodium hydroxide
p	Reference pressure
PE	Priming effect
R	Molar gas constant
$R(^{13}C/^{12}C)_{sample}$	Isotope-amount ratio
SOC	Soil organic carbon
SOM	Soil organic matter
$SrCl_2$	Strontium chloride
$SrCO_3$	Strontium carbonate
T	Reference temperature
v	Volume
VPDP	Vienna Pee Dee Belemnite
$X(^{13}C)_{sample}$	Atomic fraction
$xE(^{13}C)_{sample}$	Excess-isotopic-amount

1 Introduction

Northern peatlands cover only 3% of the global land area while storing about 30% of the global soil organic carbon (SOC) pool (Gorham, 1991). At the same time, peatlands provide various ecosystem services. One such service is climate regulation through SOC sequestration as well as water storage regulation (Ferré et al., 2019). The high-standing water table in peatlands prevents organic matter decomposition due to anoxic conditions, leading to the accumulation of partially decomposed material as SOC (Gorham, 1991; O'Kelly & Pichan, 2014). The accumulation of partially decomposed material results in peat soils being an effective carbon dioxide (CO_2) sink but also a methane (CH_4) source (Lai, 2009). These functions are increasingly vulnerable to climate change and anthropogenic pressures, potentially creating positive feedback to the climate system (Lai, 2009; Turetsky, Kotowska, Bubier, Dise, et al., 2014; Updegraff et al., 2001).

Peatlands are threatened by humans and their different land uses (Ferré et al., 2019), with over 50% of European peat soils being degraded through drainage for land use as agriculture, forestry or mining (Joosten, 2010). For agriculture, peatlands are drained to create conditions suitable for crop growth (Ferré et al., 2019). However, drainage shifts the anaerobic conditions to aerobic, which triggers the increased decomposition of peat (Bader et al., 2018). In aerobic soil respiration, microorganisms oxidize organic matter in the presence of oxygen, producing CO_2 (Bridgham & Richardson, 1992). On the other hand, in saturated (anoxic) conditions, fermentation converts organic matter into short-chain organic compounds, acetate and CO_2 (Brooks-Avery et al., 2003; Metje & Frenzel, 2007). CO_2 and acetate provide the basis for methanogenesis, which is further processed to CH_4 and water (Bridgham et al., 2013; Brooks-Avery et al., 2003; Galand et al., 2005; Metje & Frenzel, 2007). Lowering the water table increases decomposition through aerobic respiration, potentially reducing SOC (Joosten & Couwenberg, 2008). Thus, peatland drainage can have unclear effects on the total greenhouse gas budget, increasing CO_2 (Krüger et al., 2015) and decreasing CH_4 (Lai, 2009).

Fine-root growth can also increase strongly with a lower water table and drier soil conditions (Malhotra et al., 2020). This could be because the roots have more aerobic space in the peat, allowing them to spread more widely or due to increased root growth to adapt to moisture limitation (Malhotra et al., 2020). In agriculture, when plants are harvested aboveground, the roots remain in the soil and decompose. The quality of the organic matter inputs (e.g., nitrogen content or carbon to nitrogen ratio) heavily influences decomposition rates (Mao et al., 2018;

Melillo et al., 1984). High-quality plant inputs with high lignin and cellulose content, such as roots, are more resistant to decomposition and contribute to organic matter accumulation (Coûteaux et al., 1995). The quality of the plant inputs and the peat is significant for controlling carbon (C) storage and fluxes (Crow & Wieder, 2005). However, the role of plants in peatland C cycling and storage is not fully understood yet. Furthermore, it is difficult to distinguish between the heterotrophic (microbial) amount and the autotrophic (root-related) part of soil respiration (Crow & Wieder, 2005).

Another reason root inputs are essential to peatland SOC is the potential priming effect (PE) that root inputs can have on the rhizosphere microbes. The PE refers to the reduction and increases rate of decomposition of soil organic matter (SOM) due to adding fresh root litter inputs (Thiessen et al., 2013). There are two different types of PE: positive and negative. A positive PE is when the addition of root litter inputs accelerates the SOM mineralization, and if less SOM is mineralized after adding root litter, it is a negative PE (Kuzyakov et al., 2000). Positive priming occurs because when plant material is broken down, glucose is released, which stimulates microbial activity and decomposition (Kuzyakov et al., 2000). However, the abiotic and biotic drivers of priming are not fully understood (Thiessen et al., 2013).

In this thesis, I investigated the fate of C from root-derived plant litter in degraded peatlands under different litter and moisture treatments. I mixed different amounts of highly ^{13}C labelled root litter (>2 atom% ^{13}C) with degraded peat under high and low water table conditions. I aimed to simulate degraded peatlands used for agriculture and have a lower water table, as well as degraded peatlands re-saturated with water. The PE, in combination with labelled plant material, made it possible to distinguish between decomposition from the native soil material and the labelled root litter material. I investigated three research questions. 1) How does root litter quantity influence the fluxes of CO_2, CH_4, and the C content of peat soils? With more root litter inputs, I hypothesize that more C is stored in the soil, but more CO_2 and CH_4 are also released proportionally. 2) How does the interaction between root litter amounts and water treatments influence the magnitude of the PE in peat soil? I expect a higher CO_2-C PE in the field moist treatments but a higher CH_4-C PE in the saturated treatments. 3) What is the impact of the water table on CH_4 and CO_2 production at different litter amounts? I expect that at higher water tables, relatively more CH_4 is produced than at lower water tables. With this experiment, I quantify how much CO_2 and CH_4 are produced under different root litter and moisture conditions, which in turn are essential for quantifying the future global warming or cooling potential of peatlands.

2 Materials and methods

2.1 Incubation preparation

The peat samples were from the Agroscope study site in the Swiss Rhine Valley, located in Rüthi SG (47°17’ N, 9°32’ E) at an elevation of 425 m. The field site is a drained fen with a peat thickness of about 10 m (Wang et al., 2022). The climate is temperate and moist, with a mean annual temperature of 10.6 °C and a mean annual precipitation of 106 mm (from 1990 – 2020) (MeteoSchweiz, 2023). The field has an integral drainage system with a pump built in 1973. The drainage pipes have a depth of 1 m, and between the pipes is a distance of 14 m (Wang et al., 2021). After getting an overview, a profile was dug in the peat for three different spots with a depth of 40 cm at each spot. The profile was divided into different depths (0-10 cm, 10-20 cm, 20-30 cm, and 30-40cm). From each depth, a soil core was taken with a volume of 100 cm3 (for the bulk density) and peat samples (10x10x10 cm) for the incubation.

For the incubation, the peat samples between a depth of 10 and 30 cm were used because this is where most of the roots and fine roots grow. Additionally, by using the deeper parts, the surface disturbance between 0-10 cm was avoided. These samples were sieved to a size of 2 mm and mixed in a big box. All the samples were stored field moist in the fridge before the incubation. Then, some water was added to keep the moisture, and the pre-incubation was at 25°C for ten days.

The roots of ryegrass (Lolium Perenne L.) were used for the experiment. The plants of these roots grew under continuous enriched 13C conditions (10 atom%) with the help of Multi-Isotope Labelling in a Controlled Environment (Studer et al., 2017). Through this procedure, the plants and their roots were labelled. These roots were washed fresh to remove the growing media (sand mixed with 5-10 weight% commercial potting soil), dried (40°C) and cut into small pieces for homogenization.

2.2 Experimental set-up

Five root treatments at two different water treatments and one control at each water treatment with four replicates each were used for the experiment. Thus, five different root amounts, two different water saturations and controls for each were used (Table 1, for more details Table A1). The specific amount of root litter per treatment was weighed into the beakers, followed by the exact amount of peat (30 g of dry peat). The roots and the soil were mixed for 45 seconds.

The mixing was also done with the controls for having the same disturbance in each sample. Before, 6.4 ml water was added to reach the original field moisture. In the fully saturated peat treatments, 150 ml of water was carefully added after mixing the peat with the roots. The beakers were placed within airtight 1.8 l jars. Therefore, I always used the same minimum time of 15 min between mixing and starting the incubation.

Table 1. The different amounts of roots used for the experiment and their C amount per soil mass. Treatment one to five were the field moist treatments, and six to ten were the saturated treatments. Root amounts were derived from a peatland warming experiment where five different temperature levels resulted in different root biomass (Malhotra et al., 2020).

Treatment no.	Root amount [mg root/g soil]	Root C amount [mg root C/g soil]
1 & 6	2.3	0.82
2 & 7	4.1	1.48
3 & 8	5.8	2.09
4 & 9	7.6	2.74
5 & 10	9.3	3.35

A 1 M NaOH solution was prepared to trap the CO_2 during the incubation, according to (Wollum & Gomez, 1970). The NaOH solution (30 ml) was then placed in the incubation jar in a brown vial (hereafter, NaOH traps). Additionally, clear vials with water were placed in the incubation jars to keep the headspace moisture within the jars constant.

After putting everything in the jar, they were closed, and the septum was checked for the last time. This procedure was repeated for each treatment and its replicates separately. This was necessary to have the same artificial disturbance in each replicate. In the end, they were incubated at 25°C in the dark for the next four months using closed incubators (MIR-554-PE, Panasonic Healthcare Co., Japan).

Figure 1: The final set-up. On the left side, the field moist treatment and on the right side, the saturated treatment. Brown vials with the NaOH solution and clear vials with water.

2.3 Data collection

During the incubation, gas samples were taken at six predefined time points (day no. 1, 3, 10, 22, 60, 120), and the NaOH-Traps were changed.

The gas samples were taken with a 20 ml syringe through the septum in the jars. The 20 ml gas sample was put into a vacuumized 12 ml exetainer. Two gas samples for each treatment were taken. It is essential to mention that the gas samples must be taken before changing the NaOH traps; otherwise, the air within the big jar is mixed with the ambient air.

As a next step, the NaOH traps were replaced. Each time, a new 1 M NaOH solution was prepared. This procedure always happened in the same order to ensure that gas sampling was done before the incubation jar headspace was mixed with ambient air.

2.4 Laboratory analysis

The gas samples within the exetainer were measured directly with a gas analyzer for total CH_4 and CO_2 as well its $\delta^{13}C$ values relative to international Vienna Pee Dee Belemnite (VPDB) standard (G2201-i Analyzer, Picarro, Santa Clara, USA) at the CEREEP-ECOTRON IDF. The measurement took place for one minute to get a stable signal for the average and standard deviation values for total CH_4 and $\delta^{13}C$. The lower limit of the analyzer for CH_4 measurements in [ppm] was 1.8 ppm. Changes in the electrical conductivity of the NaOH traps measured the total respired CO2. The $\delta^{13}C$ of the CO_2 was determined based on Harris et al. (1997). For this,

a 1 M $SrCl_2$ solution was used to precipitate of $SrCO_3$ in a reaction of the NaOH-traps. Therefore, 2.5 ml of the NaOH trap solution was mixed with 5 ml of the $SrCl_2$ solution and centrifuged. After decanting, the participated $SrCO_3$ was dried at 50°C. The dried $SrCO_3$ was directly analyzed by dry combustion module cavity ring-down spectroscopy system in a G2101-i Analyzer (Picarro, Santa Clara, USA) to quantify the $\delta^{13}C$ signal. Additionally, the C amount of the peat, roots and their $\delta^{13}C$ before and after the incubation was measured the same way with the same device as the dried $SrCO_3$.

2.5 Calculations

Obtaining respired CO2-C from NaOH traps

An electrical conductivity meter (914 pH/Conductometer, Metrohm, Herisau, Switzerland) was used to measure the electrical conductivity of the 1M NaOH traps in units of [mS cm-1]. The device itself corrected the conductivity, depending on the temperature, directly to 25°C. This procedure is based on Wollum & Gomez (1970).

The concentration of CO_2 ($[CO_{2;NaOH}]$) trapped as $NaCO_3$ was determined in units of [mg ml-1], using a calibration obtained by Abiven & Andreoli (2011) and is calculated as follows:

$$CO_{2;NaOH} = -0.168 * EC_{NaOH;Sample-corrected} + 28.639$$

Where $EC_{NaOH;\ sample\text{-}corrected}$ is the electrical conductivity of the NaOH trap [mS cm-1] which is corrected to 25°C. The total respired CO_2-C (CO_2-$C_{\ total}$) in units of mg was then calculated as

$$CO_2 - C_{total} = CO_{2;NaOH} * v_{NaOH} * 0.2729$$

where v_{NaOH} is the volume of the NaOH trap [ml], and the mass fraction of C is considered. With that, I got the total CO_2-C_{total} in [mg].

Converting the CH_4 data [ppm] into [mg]

The CH_4 samples, which were the cumulative amount of CH_4 between the time steps with the assumption of no loss, were measured in [ppm]. To convert the units of the data to [mg], the CH_4 [ppm] is converted into CH_4-C concentration [mg/m^3] with the help of the ideal gas law as:

$$CH_4 - C\ [mg/m^3] = (0.1 * M * p * X_i) / (R * T)$$

Whereas CH_4-C is the concentration, M is the molar mass [g/mol], p is the reference pressure [mbar], and X_i is the concentration in [ppm]. The whole part is divided through the molar gas constant [R = 8.314472 kJ/kmolK] and the reference temperature T in [K].

With getting the CH_4-C concentration in [mg/m^3], it is possible to get the CH_4-C amount in [mg]:

$$CH_4 - C\ [mg]\ =\ CH_4 - C[mg/m^3]/V[m^3]$$

whereas the CH_4-C is the concentration, which was calculated before, while the V is the volume of each jar in [m^3]. Now, I got the CH_4-C in [mg] as well.

Root-derived (^{13}C labelled) material

The root-derived C in the respired CO_2-C was calculated as in (Coplen, 2011) with the atomic ^{13}C fraction. Exemplarily, the calculations are only shown for the CO_2-C, but it works the same for CH_4-C. Using R(^{13}C/^{12}C)VPDB = 0.01118, the isotope-amount ratios R(^{13}C/^{12}C)$_{sample}$ was calculated as

$$R(\ ^{13}C/\ ^{12}C)_{sample}\ =\ (CO_2 - C\ )/\ 1000) + 1) * R(\ ^{13}C/\ ^{12}C)_{VPDP}$$

Further, the atomic fraction x(^{13}C)$_{sample}$ was calculated as follows:

$$x(\ ^{13}C)_{sample}\ =\ R(\ ^{13}C/\ ^{12}C)_{sample}\ /\ (1 + R(\ ^{13}C/\ ^{12}C)_{sample}$$

Further, the excess-isotopic-amount xE(^{13}C)$_{sample}$ was calculated:

$$x(\ ^{13}C)_{sample}\ =\ x(\ ^{13}C)_{sample}\ -\ x(\ ^{13}C)_{control}$$

The x(^{13}C)$_{control}$ is the atomic fraction of the soil, SOC fractions and the respired CO_2 of the treatments without $^{13}C_{roots}$. Additionally, the proportion of root-derived C, f_{root}, for each sample was calculated as follows:

$$f_{root}\ =\ xE(\ ^{13}C)_{sample}\ /\ xE(\ ^{13}C)_{root}$$

Thereby, the xE(^{13}C)$_{root}$ is the excess-isotopic-amount fraction of the root for each treatment. Using the f_{root} of each sample, it is possible to determine the absolute amount of root-derived C in both the respired CO_2/CH_4 and the SOC fractions. The amount of native SOC in each sample was calculated as the difference between the total measured C and the corresponding root-derived C.

From the above CO_2 and CH_4 respiration terms, I calculated various specific respiration proxies to understand the mechanisms and relative contributions of root C to soil pools and fluxes. These proxies are detailed in Table 2.

Table 2. Names, calculations, units and ecological meaning of each respiration proxy used in this paper. C_{native} is the native-derived C which comes from the C pool that was already present in the soil. C_{root} is the root-derived C which comes from the added root amount. The sum of the native and root C is C_{total}. Partially, the mean value was calculated from the replicates for these values (n = 4).

Row	Name	Calculation	Unit	Ecological meaning
(1)	Total specific CO_2-C respiration	(mg CO_2-C_{total}/g C $_{total}$)	mg CO_2-C_{total}/g C_{total}	Total CO_2-C or CH_4-C emitted from incubation standardized by the total C in the soil
	Total specific CH_4-C respiration	(mg CH_4-C_{total}/g C $_{total}$)	mg CH_4-C_{total}/g C_{total}	
(2)	Root specific CO_2-C respiration	(g CO_2-C_{root}/g C_{root})/100	%	Fraction of root-derived CO_2-C or CH_4-C emitted from incubation standardized by the root C
	Root specific CH_4-C respiration	(g CH_4-C_{root}/g C_{root})/100		
(3)	Native C specific CO_2-C respiration	(g CO_2-C_{native}/g C_{native})/100	%	Native-derived CO_2-C or CH_4-C emitted from incubation standardized by the native C
	Native C specific CH_4-C respiration	(g CH_4-C_{native}/g C_{native})/100		
(4.1)	CO_2 priming effect	Treatment$_{(\text{Native OC specific CO2-C respiration})}$ - Control$_{(\text{Native OC specific CO2-C respiration})}$	mg native C specific CO_2-C or CH_4-C respiration/g soil	The amount of native-derived CO_2-C or CH_4-C which was respired through the additional roots
(4.2)	CH_4 priming effect	Treatment$_{(\text{Native OC specific CH4-C respiration})}$ - Control$_{(\text{Native OC specific CH4-C respiration})}$		
(5)	Relative CO_2 priming effect	(4.1) /Control$_{(\text{Native OC specific CO2-C})}$*100	%	The relative amount of native-derived CO_2-C or CH_4-C which was respired through the additional roots
	Relative CH_4 priming effect	(4.2) /Control$_{(\text{Native OC specific CH4-C})}$*100		

(6)	Theoretical pool of remaining roots	(mg $C_{root\ start}$ - (mg CO_2-C_{root} + mg CH_4-C_{root}))	mg C	Root C which was remaining in the end of the incubation in the soil

2.6 Statistical analysis

All the variables in Table 2 were used in the analysis. The statistical analyses and calculations were performed using RStudio 2023.03.0 (R Core Team, 2023).

Given the full factorial design of my experiment, most statistical models were linear regressions with the function y~ f(water treatment, root input, water treatment x root input) unless otherwise stated. This allowed me to determine the effect of different root biomass and different water levels on the C fluxes of degraded peat. In addition, a Wilcoxon Rank test was performed to test whether the different PEs were significantly different to the control.

3 Results

3.1 Root litter input impacts on carbon fluxes

Different amounts of root litter input had a significant effect on soil respiration. With more root litter, the amount of respired CO_2-C and CH_4-C increased (Figure 2). There was no detectable CH_4-C respiration within the field moist treatments. In general, CO_2-C respiration was lower in the saturated treatments. This corroborated my hypothesis that more CO2 and CH4 were released with more root litter.

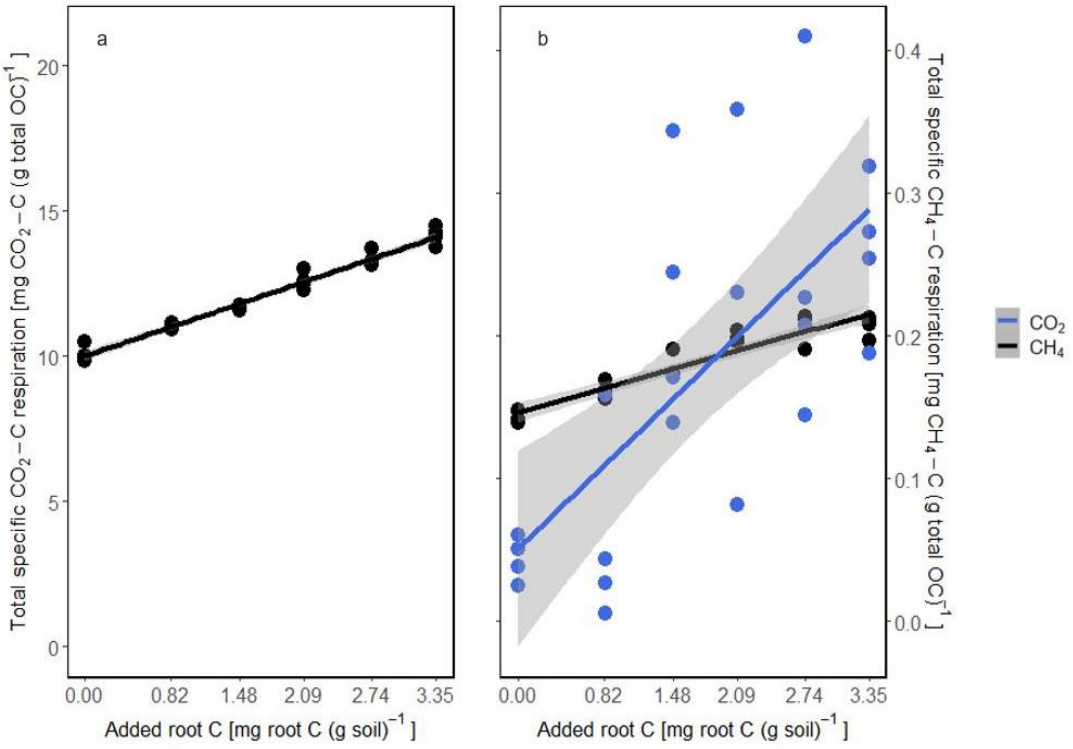

Figure 2: Different root C amount influences the C fluxes in both field moist (a) and saturated conditions (b) for CO_2 (left axis) and CH_4 (right axis). Significant trends (Table 3) were seen across all the respirations. No detectable CH_4 was observed in the field moist treatment.

In addition to the significant effects of root litter amounts on soil respiration, specific respiration indices (Table 2) were significantly related to root-derived C (Figure 3). The C_{root} was proportionally more respired than C_{native} for CO_2 and CH_4. In the field moist conditions, the respiration increased linearly with the different added root C amounts. In the saturated conditions, root litter amount and CH_4-C_{root} flux were non-linearly related (quadratic regression $R^2 = 0.2775$, $p = 0.03415$).

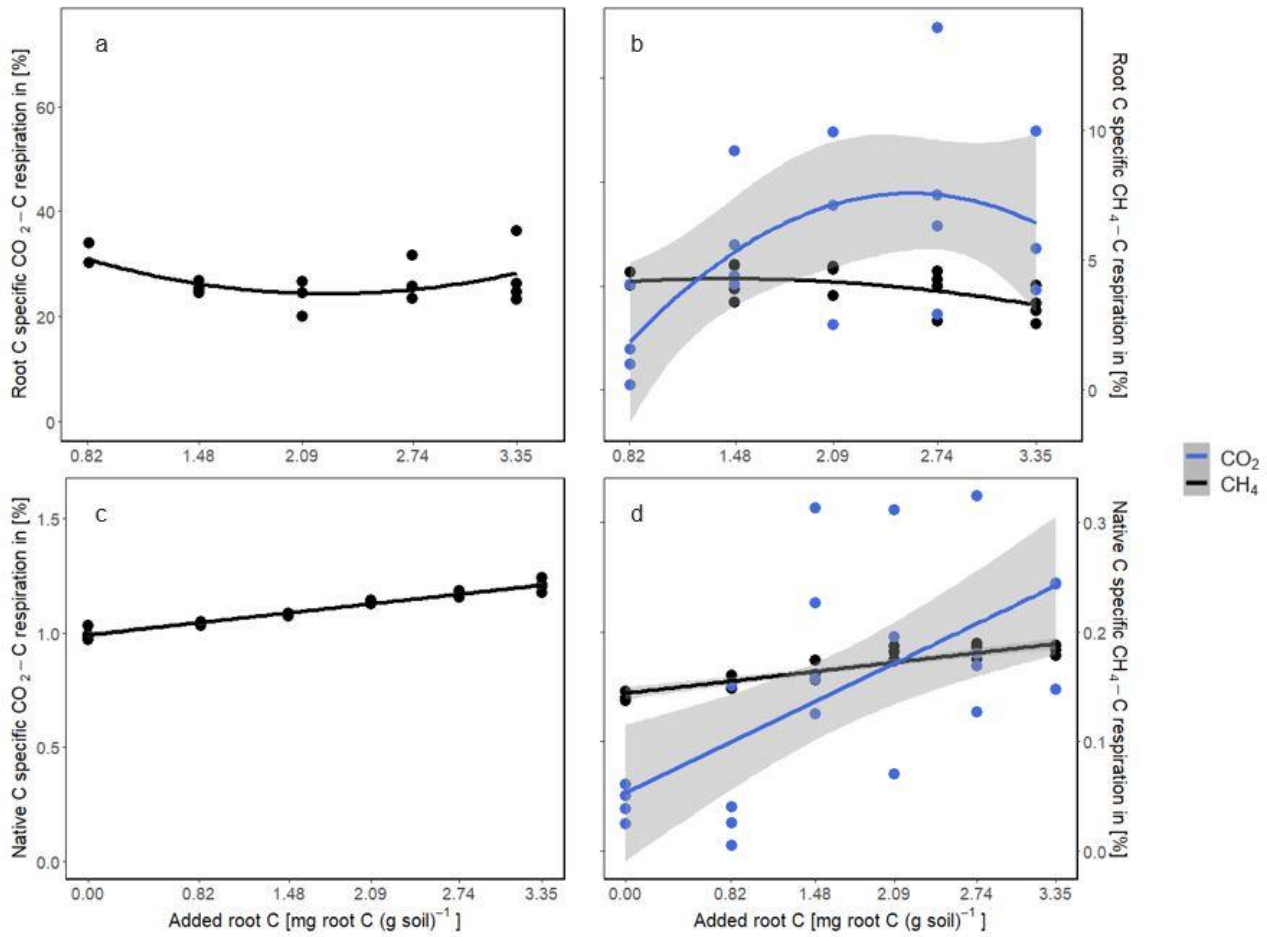

Figure 3. Root amount influences C fluxes in both field moist (a and c) and saturated conditions (b and d) for CO_2-C (left axis) and CH_4-C (right axis). Significant trends (Table 3) were seen across the specific respiration types: native-derived respiration normalized by native C (c and d) and root-derived respiration normalized by root C (a and b).

The root amount added had a similarly significant relationship to native and total respired C (Figure 3c and 3d; Table 3 for the statistical output). Both fluxes were statistically significant, which corroborates my hypothesis that with more root litter, more CO_2 and CH_4 are released.

Table 3. Multiple linear regression models outputs for a range of C flux and respiration proxies (see Table 2 for proxy definition).

	Model-adjusted R^2	Slope estimate	Standard Error	Degrees of freedom of denominator	p-value
Total specific CO_2-C respiration	0.9693				< 0.0001
- **Intercept**		10.12	0.1396	72.473	<0.0001
- **Fine root mass**		0.01476	0.0008067	18.301	<0.0001
- **Water amount**		-0.01794	0.001261	-14.223	<0.0001
- **Interaction**		<-0.0001	<0.0001	-2.017	0.0498
Total specific CH_4-C respiration	0.4617				0.0002176
- **Intercept**		0.0509180	0.0330912	1.539	0.138806
- **Fine root mass**		0.0008546	0.0001917	4.458	0.000218
Root-specific CO_2-C respiration (quadratic regression)	0.5952				<0.0001
- **Intercept**		417.6	53.26	7.841	<0.0001
- **Fine root mass quadratic**		0.004963	0.001860	2.668	0.01201
- **Water amount**		-1.489	4.752	-3.133	0.00377
- **Interaction**		< -0.0001	<0.0001	-2.701	0.01110
Root-specific CH_4-C respiration (quadratic regression)	0.2775				0.03415
- **Intercept**		-5.0426092	4.2823285	-1.178	0.257
- **Fine root mass quadratic**		-0.0002777	0.0001619	-1.716	0.107
Native OC-specific CO_2-C respiration	0.9696				<0.0001
- **Intercept**		10.03	0.1114	90.067	<0.0001
- **Fine root mass**		0.007830	0.0006319	12.391	<0.0001
- **Water amount**		-0.01806	0.001005	-17.962	<0.0001
- **Interaction**		<0.0001	<0.0001	0.341	0.735
Native OC-specific CH_4-C respiration	0.3853				0.001223
- **Intercept**		0.0532273	0.0300164	1.773	0.09141
- **Fine root mass**		0.0006797	0.0001806	3.763	0.00122
CO_2 Priming effect (quadratic regression)	0.9903				<0.0001
- **Intercept**		10.05	0.1549	64.881	<0.0001
- **Fine root mass quadratic**		0.001853	0.007708	0.240	0.8180
- **Water amount**		-0.01941	0.001400	-13.865	<0.0001
- **Interaction**		-0.0001114	<0.0001	-1.600	0.1606
CH_4 Priming effect (quadratic regression)	0.08565				0.3559
- **Intercept**		0.0465233	0.0409284	1.137	0.282
- **Fine root mass quadratic**		0.0008764	0.0009055	0.968	0.356

3.2 Impacts of different root litter amounts and water tables on the priming effect

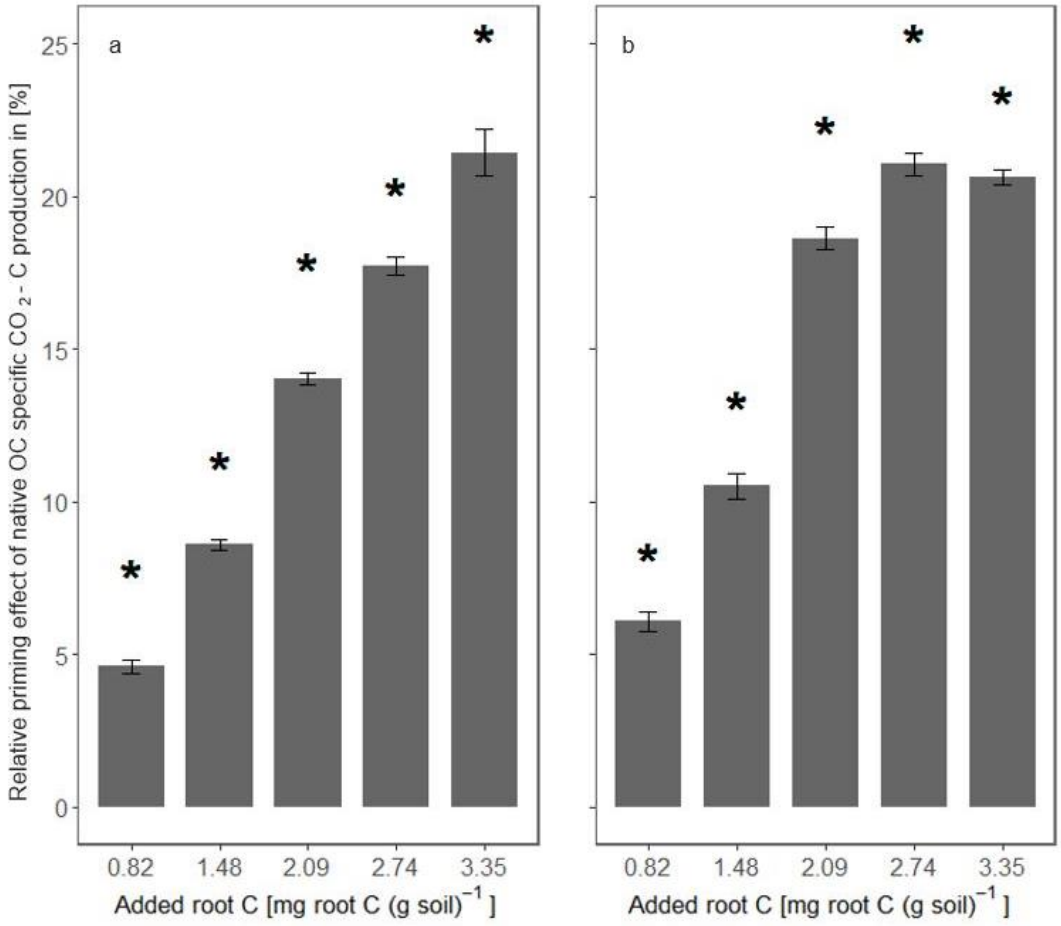

Figure 4. The CO_2-C PE as a function of different amounts of root C inputs for the field moist (a) and saturated treatments (b). The statistical significance of the difference between the control (0 g root amount added; shown in Figure 2) and the different root treatments were tested using a Wilcoxon test (significance level: * is when p <0.05).

Building upon the observations from the specific respiration analyses (Figures 2 and 3), the investigation of the PE provides additional insights into the relationship between root litter amounts and soil respiration. The PE in the different gas fluxes was positive, i.e., more respiration was produced adding root litter. The CO_2-C PE increased with increasing root C amounts in both treatments (Figure 4). Interestingly, unlike the linear CO_2-C PE in the field moist treatments, the CO_2-C PE did not show a linear PE under saturated conditions. There, the PE decreased with the last added root C amount. Each root litter C amount added was significantly different from the control (Wilcoxon test $p < 0.05$; Table 4).

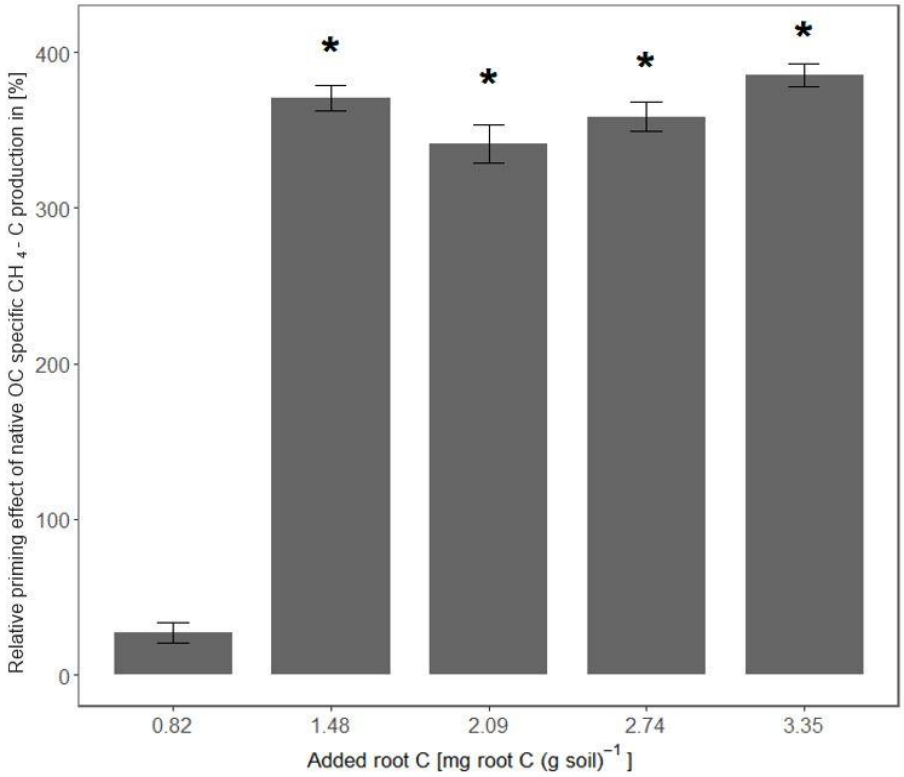

Figure 5. The CH_4-C PE in the saturated treatments results from the different root inputs. The statistical significance of the difference between the control (0 g root amount added; shown in Figure 2) and the different root treatments were tested using a Wilcoxon test (significance level: * is when $p < 0.05$).

While the higher added root amounts showed significant PE, the lowest root amount (0.82 mg root C/g soil) showed minimal PE for CH_4. With increasing root-added amounts, I observed a remarkable increase in a positive PE of more than 350% for CH_4, even though the positive PE amounts stayed similar for the four higher root-added amounts (Figure 5). Each root litter C amount added was significantly different from the control except for the first added amount (Wilcoxon test $p < 0.05$; Table 4).

In the field moist treatments, the relative CO_2-PE was stronger than the relative CH_4-PE (because there was no detectable CH_4). Conversely, in the saturated treatment, the relative CH_4-PE was much stronger than the relative CO_2-PE. This corroborates my second hypothesis that CO_2-PE is high in the field moist treatments, while CH_4-C PE is high in the saturated treatments.

Table 4. Wilcoxon rank sum test outputs for differences between the control and the priming effects of the specific root amounts (n=4).

Tested between	Water-treatment	CO_2-C_{native} respiration p-value	CO_2-C_{native} W-value	CH_4-C_{native} respiration p-value	CH_4-C_{native} W-value
Control and 2.3 g root treatment	field moist	0.0771	1	-	-
	saturated	0.03398	0	0.7728	9
Control and 4.1 g root treatment	field moist	0.02092	0	-	-
	saturated	0.02092	0	0.02092	0
Control and 5.8 g root treatment	field moist	0.03389	0	-	-
	saturated	0.02092	0	0.03389	0
Control and 7.6 g root treatment	field moist	0.02092	0	-	-
	saturated	0.02092	0	0.02092	0
Control and 9.3 g root treatment	field moist	0.02092	0	-	-
	saturated	0.02092	0	0.03389	0

Peat soil sequestered root C in both field moist and saturated conditions. The remaining root C in the soil increased linearly as more root C was added (Figure 6). This supports my first hypothesis that more C is stored in the soil with more root litter inputs. Interestingly, the loss of CO_2-C_{root} in the field moist treatment was linear, while in the saturated treatment, it flattened in the highest root C inputs. Furthermore, the CH_4-C_{root} did not compensate for this CO_2-C_{root} flattening.

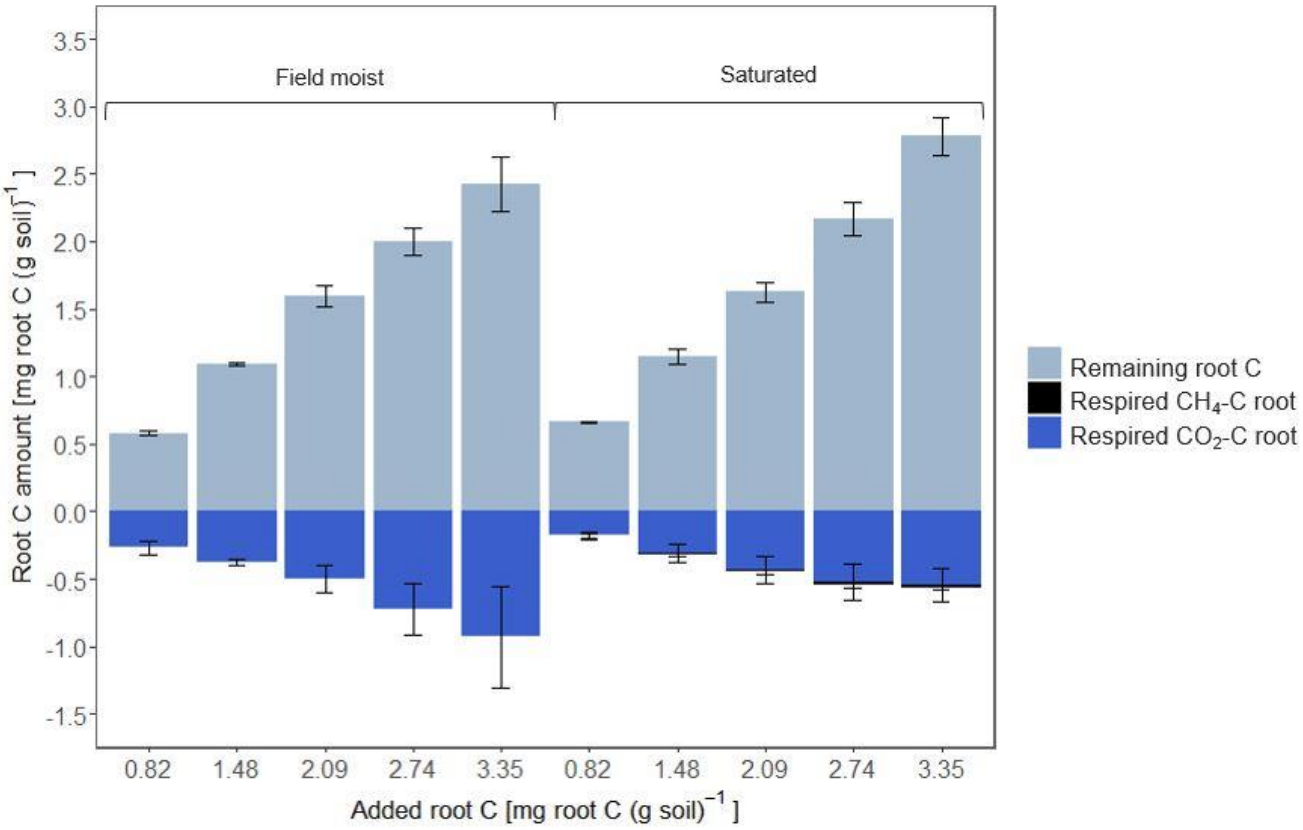

Figure 6. Theoretical root C balance shows the relationship between added root C, remaining root C and loss of root C through soil respiration. The negative values represent the loss of root C through soil respiration, while the positive values represent the remaining root C amount.

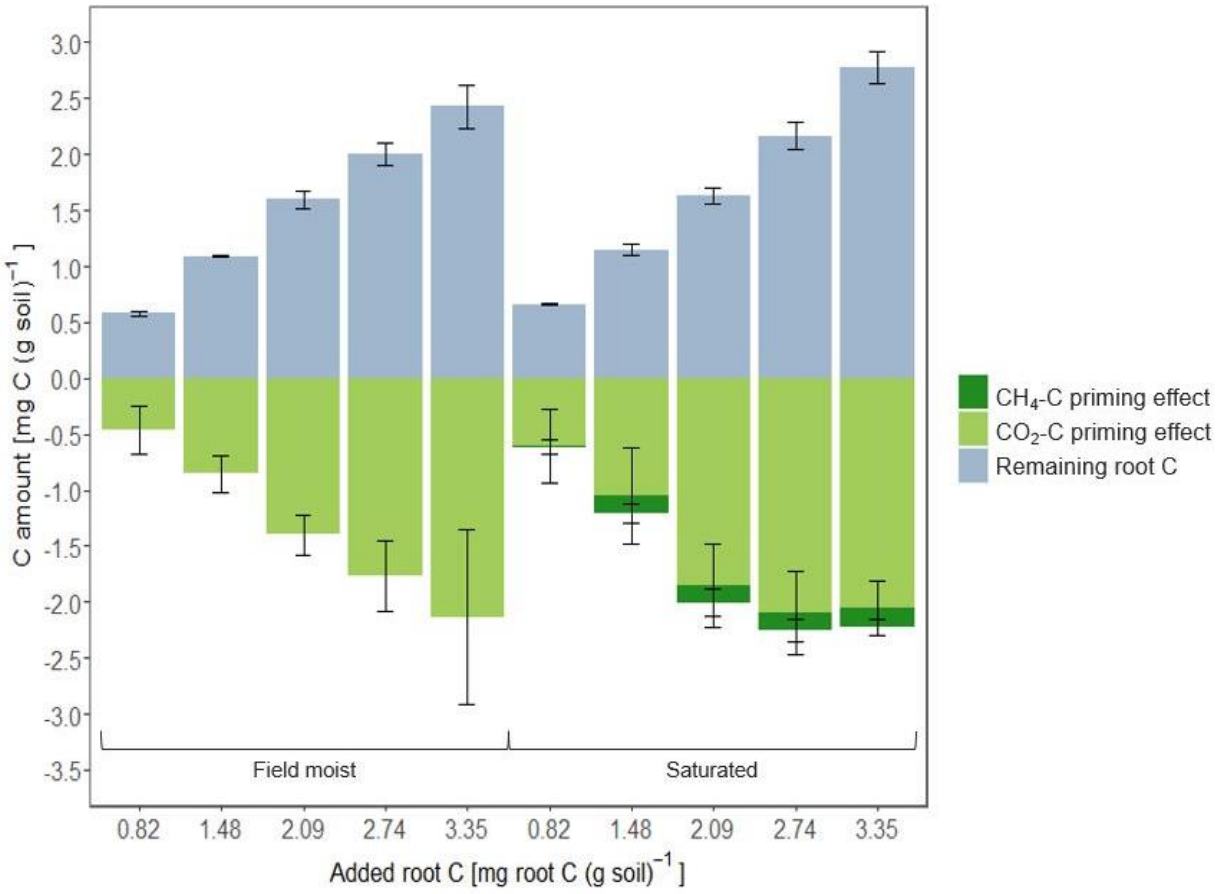

Figure 7. The relationship between the remaining roots and PE. The remaining root C in soils are shown above the zero line and the PE are shown below the zero line. In the field moist treatment (left), there is a linear increase for remaining root C and a linear decrease of PE, which consists only of CO_2-C. Conversely, in the saturated treatments (right), the PE is predominantly observed as CO_2-C_{native}, while the CH_4-C_{native} contributions are small.

The linear increase in the remaining root C indicated the potential for enhanced C sequestration, while PE increased with increasing root C input. The CO_2-C PE increased linearly in the field moist conditions, whereas it plateaued in the saturated conditions (Figure 7, Table 3). The CH_4-C PE compensated for some of the plateaus of the CO_2-C PE. Despite the PE, slightly more root C was retained in the soil than native C respired. In other words, C was stored in the peat, and the peat acted as a C sink.

3.3 Impact of different water treatments on methane and carbon dioxide production at different amounts of litter

The saturated treatments produced more CH_4-C than the field moist treatments, and conversely, the CO_2-C respiration was higher in field moist conditions. This supports my last hypothesis that emissions of CH_4 and CO_2 strongly depend on the water table. Multiple linear regression analysis showed significant interaction coefficients between the treatments with water and different root amount inputs (Table 2), suggesting that the relationship between water saturation, root amount input, and the various respiration proxies were not additive or independent but included complex synergistic or antagonistic interactions.

4 Discussion

I investigated how root-derived C cycles through peat soils, focusing on the response of C fluxes and stocks to varying root litter amounts and moisture availability. I found that increased root litter inputs stimulated CO_2 and CH_4 fluxes and increased SOC. Furthermore, CO_2 fluxes were higher in the field moist treatments than in the saturated peat treatments, and as expected, CH_4 fluxes showed the opposite trend. Root litter had a positive relationship with soil respiration, while moisture amount had a negative relationship with CO_2-C respiration and a positive relationship with CH_4-C soil respiration. Additionally, I observed a PE with increasing root litter amounts wherein both CO_2 and CH_4 emissions from the native soil C increased. Interestingly, the relative PE was much stronger in the CH_4 emissions than in the CO_2 emissions. My study provides some of the first evidence of how root C influences C fluxes and SOC stocks in peatlands.

4.1 Despite priming, more C is stored in the soil than respired

Root litter triggered a PE that had significant implications for greenhouse gas emissions of native-derived C, which was massively higher for CH_4-C than for CO_2-C. Nevertheless, more root-derived C was stored in the soil than respired as CO_2 or CH_4 (Figure 7). Notably, the relative PE for CH_4-C_{native} was 20 times higher than that of CO_2-C_{native}, which indicated a disproportionate impact on the additional root C on CH_4 production. A possible reason for this could be that the initial concentration of aerobic decomposer microorganisms was already very high in the degraded peatland used for agriculture, while methanogenic bacteria were relatively limited due to the aerobic conditions. It is possible that when the peat was re-saturated and roots were added, the anaerobic microbial communities were able to multiply rapidly, leading to the observed PE. However, anaerobic microbial communities seemed to need a certain level of easily degraded organic matter to multiply. This could be why there was hardly any difference between the CH_4-C PE of the control and the first root treatment. For the remaining root amounts, soil respiration increased strongly and rapidly but was similar for all root treatment levels. A similar higher CH_4 than CO_2 respiration has been observed in the past. In a experiment, peat samples from the Southern Taiga subzone were mixed with peat pore water, filled into a sealed glass bottle, and filled with nitrogen gas so no oxygen was present. The samples were incubated at 15°C for 20 days (short term run) and 142 days (long term run). Gas and liquid samples were taken during incubation, and the CH_4, CO_2 and pH values were

analyzed. They attributed their results to the near-neutral pH value and the traditional pathways of organic matter degradation (Lokshina et al., 2019). In my study, the pH of the soil and the water in the saturated treatments, were not measured but were kept consistent across treatments, at least at the initiation of the experiment. Therefore, it is difficult to draw a comparison to the mentioned study. It is also challenging to compare methanogenesis since I did not look deeper into the methane formation in this study either. However, the methanogenesis pathway can vary depending on the relative initial concentration of substrates and biomass (Lokshina et al., 2019). This could indicate that a different kind of methanogenesis occurred in the treatments with a higher positive CH_4-C PE than in the treatment with only little CH_4-C PE.

Beyond the PE, an analysis of the C storage in soil revealed that slightly more root-derived C was stored in the soil than respired. While C loss by CO_2 and CH_4 respiration ranged from 0.46 to 2.22 mg C/g soil of the added C through root litter, the additional C stored in peat ranged from 0.58 to 2.78 mg C/g soil (Figure 7). Overall, more root C was stored in the soil than native C was released.

4.2 Fresh root carbon is preferred by microorganisms over native carbon

With additional root C inputs, soil respiration increased, and in proportion, more root-derived C was released than native-derived C (Figure 3), likely because organic matter with a high nitrogen content decomposes faster and is easier to decompose than low nitrogen content organic matter (Coulson & Butterfield, 1978; Mao et al., 2018; Melillo et al., 1984). For my experiment, only deeper layers (10-30 cm) of the peat were used, which is also where the majority of the roots grow. This layer has an average nitrogen content of about 1.25 % (Wang et al., 2023). Assuming that the used ryegrass Lolium Perenne L. has a slightly higher nitrogen content, the ryegrass should decompose faster. When the incubation started, no fresh root from the field was left in the peat due to the pre-incubation. With the pre-incubation, the microorganisms degraded the remains of the easily degradable compounds of the roots from the field side. When starting the experiment, fresh roots and, with it, new easily degradable compounds were introduced. As the microorganisms prefer that, consequently, a higher proportion of C_{root} was respired than C_{native}. Since about 70-82% of the roots were stored in the soil, while 18-30% of the roots were respired, it is possible that a considerable proportion of the respired roots consisted of easily degradable components.

While my analyses focus on cumulative data from the incubation, it is worth noting that the CO_2-C_{root} proportion trends by day also provide insights into the microbial decomposition of the fresh root material (Figures A1 & A2). The system had to stabilize in the first few days because the preparation and mixing caused an artificial disturbance. Afterwards, interestingly, the treatments under saturated conditions stabilized faster than the field moist treatments. The proportion of C_{root} in CO_2-C flux plateaued after 60 days under field moist conditions but increased until day 60 and then decreased in the saturated conditions. The root-derived proportion CH_4-C trend (Figure A3) looked similar to the CO_2-C_{root} proportion under saturated conditions. The root-derived CH_4-C proportion stabilized rapidly and reached its peak on day 22, after which it dropped again. If the proportion of root C increases continuously, this would mean that an ever larger proportion of the roots would be respired, and thus hardly anything would be stored in the soil in the long term. If, on the other hand, the proportion decreases after a certain point, this means that a smaller proportion of roots is consumed, and thus a certain amount is stored in the peat as it is in this study. A general reason for the trends in Figures A1, A2, and A3 could be that the easily degradable components from the roots were used up. Thus, after 60 days, only the hard-to-degrade components would still be present, and the degradation would no longer be so fast. In field moist treatments with aerobic soil respiration, it could be that the microbial activity reached a saturation point after these days due to factors such as nutrient limitations or a shortage of oxygen (Melillo et al., 1984). This could lead to a plateau in the proportion of root-derived CO_2-C flux. Something that supports oxygen limitation is that the jars were only opened at specific time points and that there was no other oxygen exchange. This could be important, especially in the longer periods between days 20 and 60 and between 60 and 120, where the jars were never opened. However, the trend indicated that initially more of the roots were degraded, and after a certain point, it became less while proportionally more native C was respired. In the case of the CH_4 trend, there may be a different reason. Root exudates are thought to drive CH_4 production. In addition, some evidence also suggests that root exudates stimulate a priming effect, thus promoting the decomposition of poorly decomposable material (Basiliko et al., 2012; Bridgham et al., 2013). Even if I used dried roots, they may still contain traces of root exudates that can cause this effect and are also quickly consumed by the microorganisms. This, in turn, could explain the rapid increase in the proportion of root-derived C until day 22 and also the rapid decrease afterwards.

4.3 A higher water amount promotes methanogenesis, whereas glycolysis prefers it drier

As per my hypothesis, CH_4 respiration increased with soil moisture while CO_2 respiration decreased. Methanogenesis requires an oxygen-free environment, a necessary condition for the growth and metabolism of anaerobic microorganisms. Most methanogens lack metabolic strategies from the more reactive oxygen species. This is why CH_4 emissions are controlled by soil moisture (Bräuer et al., 2020). The observed increase of methanogenesis with soil moisture was already found in previous research, which showed that the water availability influenced CH_4 production positively (Bohdalkova et al., 2014; Høj et al., 2006; Macdonald et al., 1998; Yu et al., 2013). Saturated environments, such as peatlands, are well-known sources of atmospheric CH_4 due to the perfect environment for methanogenic bacteria (Kotsyurbenko et al., 1996; Turetsky, Kotowska, Bubier, & Dise, 2014). The CO_2 respiration was lower in the saturated treatments than in the field moist treatments. In a drier environment, aerobic soil respiration takes place, which is the metabolic pathway for the breakdown of organic matter into CO_2 (Bridgham & Richardson, 1992).

For the presence of CO_2 in saturated conditions, there could be one main reason. A preliminary step of methanogenesis is fermentation (Brooks-Avery et al., 2003). By fermentation, organic matter is converted into alcohol and CO_2 (Kotsyurbenko et al., 1996). Thereby, fermentation produces a lot of CO_2 (Brooks-Avery et al., 2003), and probably not all of it is used up in methanogenesis. Thus, the CO_2 could pass into the water and could be released into the air. I assume that methanogenesis was able to develop so strongly due to the high fermentation rate among other factors. However, the results clearly showed that higher water saturation promotes methanogenesis, supporting the existing literature.

4.4 Implications for carbon cycling and greenhouse gas emissions

Understanding the relationship between soil moisture and microbial metabolic processes has significant implications for global biogeochemical cycles. This information can be valuable in predicting ecosystem response to changing environmental conditions, such as agriculture. Agriculture uses peatlands in Europe mainly by draining the water for cultivation and pastures (Ferré et al., 2019). However, this lowers the water content in the soil and increases the oxygen content (Bader et al., 2018). My study showed that aerobic soil respiration increased with decreasing soil moisture while methanogenesis decreased. The contrasting response of these

metabolic processes highlighted the specific requirements of the microorganisms involved and their adaptation to different environmental conditions. As peatlands are large C sinks (Gorham, 1991), it is essential to understand the sources of peatland soil respiration.

New plant species and thus also new plant litter are introduced into the peat due to the new agricultural use. The grass I used in the study is not a species that typically occurs in a peatland. Peatlands are dominated by graminoids, dwarf shrubs, and trees (Malhotra et al., 2020; Mao et al., 2018). The new plant species also add new, different amounts of C to the soil, which I simulated in the experiment by using different amounts of root litter. I show that root additions triggered a PE. However, despite the increased C emissions, more root-derived C can be stored in the soil than native-derived C is released. It should be noted that the amount of newly stored C is minimal. Nonetheless, my study highlights the potential of root-derived C to serve as a means of long-term C sequestration. A future research challenge is to find ways to enhance the sequestration potential while mitigating the associated increase in emissions.

The results I report have implications for a broader context, especially for managing C dynamics and soil C sequestration. In principle, more CO_2-C than CH_4-C was emitted in the study. However, the positive PE due to the additional roots is massively higher for CH_4-C than for CO_2-C. This, again, can be problematic because CH_4 emissions have about 25 times the heat-binding capacity of CO_2 (Bridgham et al., 2013; Forster et al., 2007). Thus, even the smallest amounts of CH_4 can have significant impact on climate change (Figure A4). The PE of CH_4 native described in this paper raises concerns about its potential contribution to climate change because it is a potent greenhouse gas. The feedbacks of CH_4 emissions to the climate and its changes are especially challenging to predict (Bräuer et al., 2020). There are studies that say that the CH_4 increases the climate warming and therefore the temperature, but that this is coupled with a negative feedback loop. However, due to the increased temperatures, the soil moisture in peatlands decreases, which lowers the CH_4 emissions (Cao et al., 1998).

However, it must be said that the measured effect is only as strong for completely saturated peatlands. If the water table is a few centimeters below the soil surface, there is a potential CH_4 oxidation zone. This is the zone above the saturated zone that is not fully water-saturated, and there, oxygen can diffuse into the peat, which is used to oxidize CH_4 to CO_2 (Yavitt et al., 1988). In aerobic soil, up to 95% of CH_4 can be oxidized (Updegraff et al., 2001). This can be an important point in a peat area where the soil is not saturated to the surface or where the water level changes. Because as soon as some oxygen is added, the CH_4 can be oxidized, which is a

fast process, and large amounts of it can be oxidized (Sundh et al., 1994; Yavitt et al., 1988). Thus, the methane formed in the saturated soil layers could be converted to CO_2 by oxidation, making it less harmless for the climate.

In summary, the findings indicate that the addition of root litter had a significant influence on carbon cycling and greenhouse gas emissions. While methane production increased disproportionately compared to carbon dioxide, the study also revealed that more root-derived carbon was stored in the soil than respired, underscoring the potential for long-term carbon sequestration.

5. Limitations

A limitation of this study was the number of replicates of the treatments because the scatter of the CH_4 data was partly very large. With four replicates, it was difficult to assess how well the individual data points reflected the big picture with the whole process. In addition, with the gas samples collected in a vacuumized exetainer, it was not sure if the exetainer was closely sealed and if there was a vacuum. Some of the CH_4 data points had to be omitted because they were obviously not air-tight, which was also a considerable point that limited the statistical analysis. Additional replicates would have allowed me to look, for example, at the nutrient development within the soil. The additional replicates would also have allowed me to look at more data over time, such as the C amount within the peat or the water. In the study, I was only able to measure most variables before and at the end of incubation and compare them. The only thing I was able to measure over time was the respiration rates as I was able to take gas samples and replace the NaOH traps at the different time points. Moreover, that leads me to another essential limitation, I could not measure dissolved organic C for the thesis. I assume that a significant amount of C was stored in water in the saturate treatments, which was neglected in the thesis. This would strongly influence the root C balance.

A major limitation of this study was that microbial activity was not measured. Therefore, since soil respiration and stimulation of the PE are based on microbial activity, we cannot make more accurate conclusions. I can only indirectly guess how the microorganisms evolved. In addition, only a single plant type was used. In the real world, a mixture of different plants and, thus, different types of plant litter would prevail.

Lastly, given the experimental nature of incubation experiments, I can draw limited conclusions about the real environment. Such an experiment is too limited to even begin to reflect the complexity of the environment, and many inputs (e.g., temperature, saturation changes over time) were not considered. However, the experiment gives us hints on how some of the mechanisms work.

6 Conclusion

This study investigated the effects of labelled root litter amounts and water treatment (field moist, saturated) on the C cycle (especially on CO_2, CH_4 and stored C) within degraded peatlands. The first research question addressed how different quantities of root litter inputs influenced the C cycle in peatlands, which showed that with increasing root litter amount, the soil respiration for both CO_2 and CH_4 increased, and more C was stored in the soil. My second research question investigated how interactions between root litter amounts and water treatments influenced the magnitude of PE in peat soil. As hypothesized, a higher CO_2-C PE was found under field moist conditions, while a higher CH_4-C PE was found in the saturated treatments. The last research question dealt with the impact of the water table on CH_4 and CO_2 production at different amounts of litter and found that a higher water table favoured the CH_4 production and vice versa.

Although it was only an incubation experiment, it is difficult to extrapolate from a tiny jar to an entire peatland. However, this study showed how different root amounts and moisture levels can impact peatlands. Additionally, it showed that one of the big challenges is to increase the amount of new C out of roots that are stored in the peat. The results of this work should also be treated with caution due to other limiting factors. Essential variables, e.g., dissolved organic C or microbial activity, were not measured, which could further elucidate mechanisms. In addition, the experiment provided only the data for soil respiration over time. All other variables could only be measured before incubation and at the end. Thus, it is difficult to make definite statements about trends over time.

Nevertheless, my thesis provided innovative insights into how root litter amounts and water saturation affect the C cycle in peat. It provided further insight into how PE occurs in organic soils. By filling gaps in our understanding of C dynamics, this study improves our knowledge of ecosystem functioning and how we can better assess agricultural influences on peatlands.

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8. Appendix

8. 1 Proportion of root-derived CO2-C over time

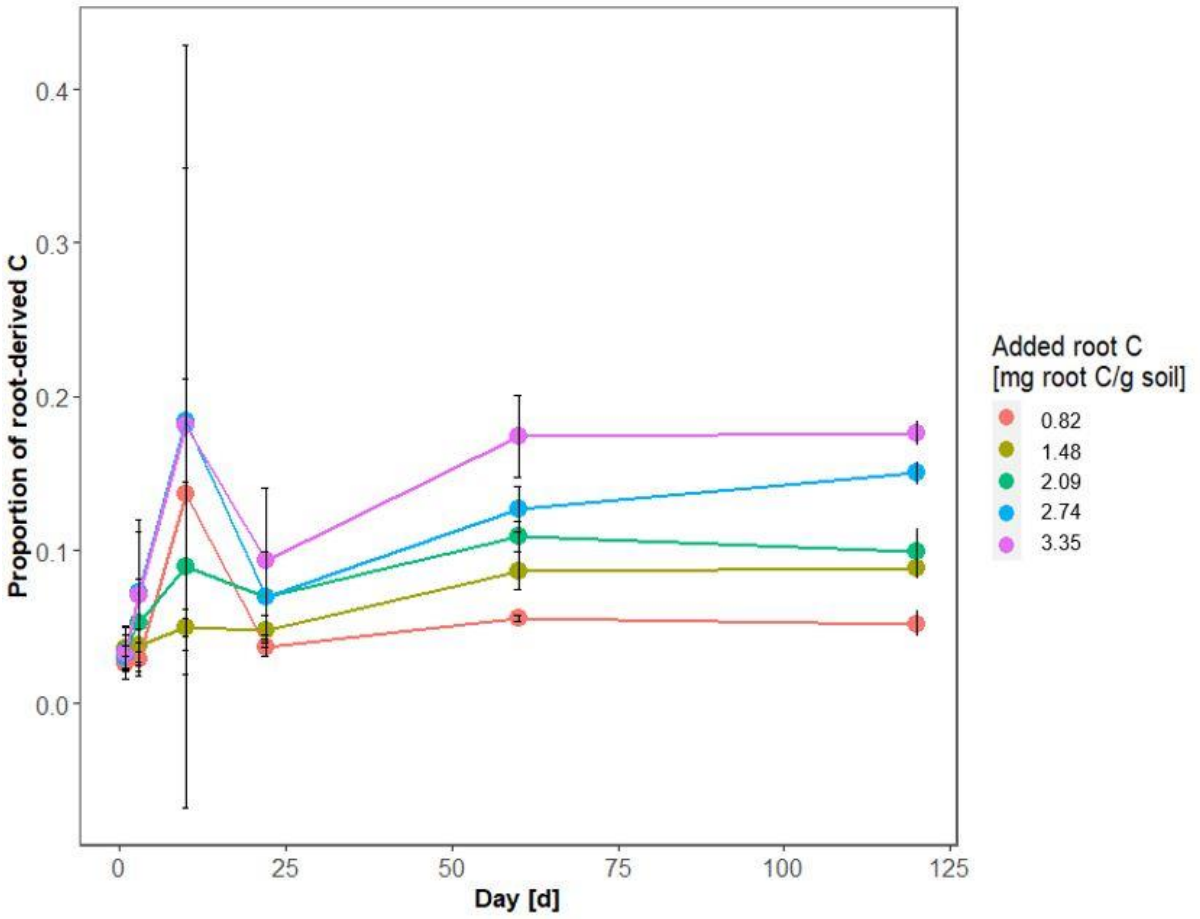

Figure A1. Proportion of root-derived CO_2-C over time of the field moist treatments. During the first few days, the system, i.e., the microorganisms, had to stabilize first. By mixing the peat with the roots, a lot of oxygen was mixed into the soil, which resulted in an artificial disturbance. That is why you can clearly see the peak at day 10 and the high standard error. From day 22 we can assume that the system has stabilized, which can be seen in the trend. The proportion of root-derived C still increases a bit and then stabilizes for most of the treatment. In the treatment with 2.74 mg root C/g soil, it increases a little further, while the treatment with 2.09 mg root C/g soil decreases a little.

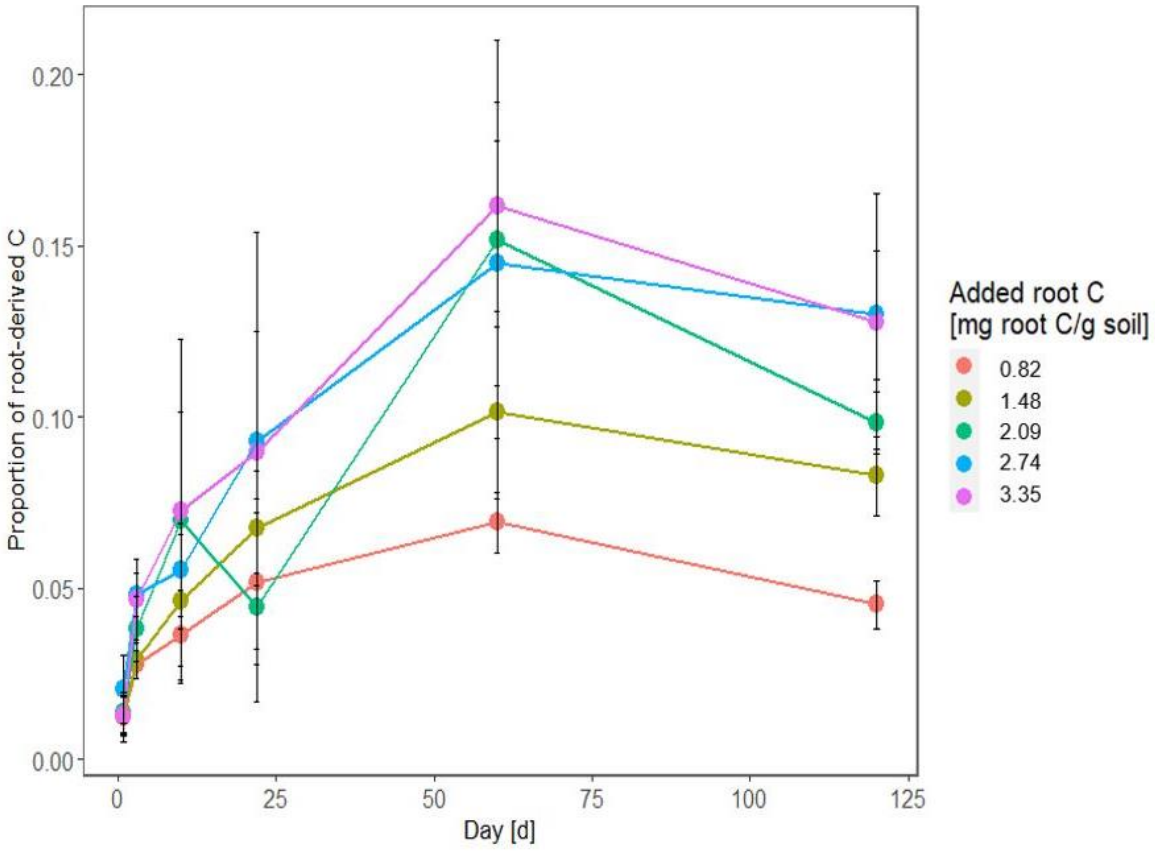

Figure A2. Proportion of root-derived CO_2-C over time of the saturated treatments. During the first few days, the system, i.e., the microorganisms, had to stabilize first. Interestingly, it looks like most saturated treatments recover more quickly from the artificial influence than the field moist treatments in Figure A1. Here, the proportion increases until day 60 and then drops again for all the treatments. Note that the proportion of root-derived CO_2-C is somewhat less than for the field moist treatments.

8.2 Proportion of root-derived CH4-C over time

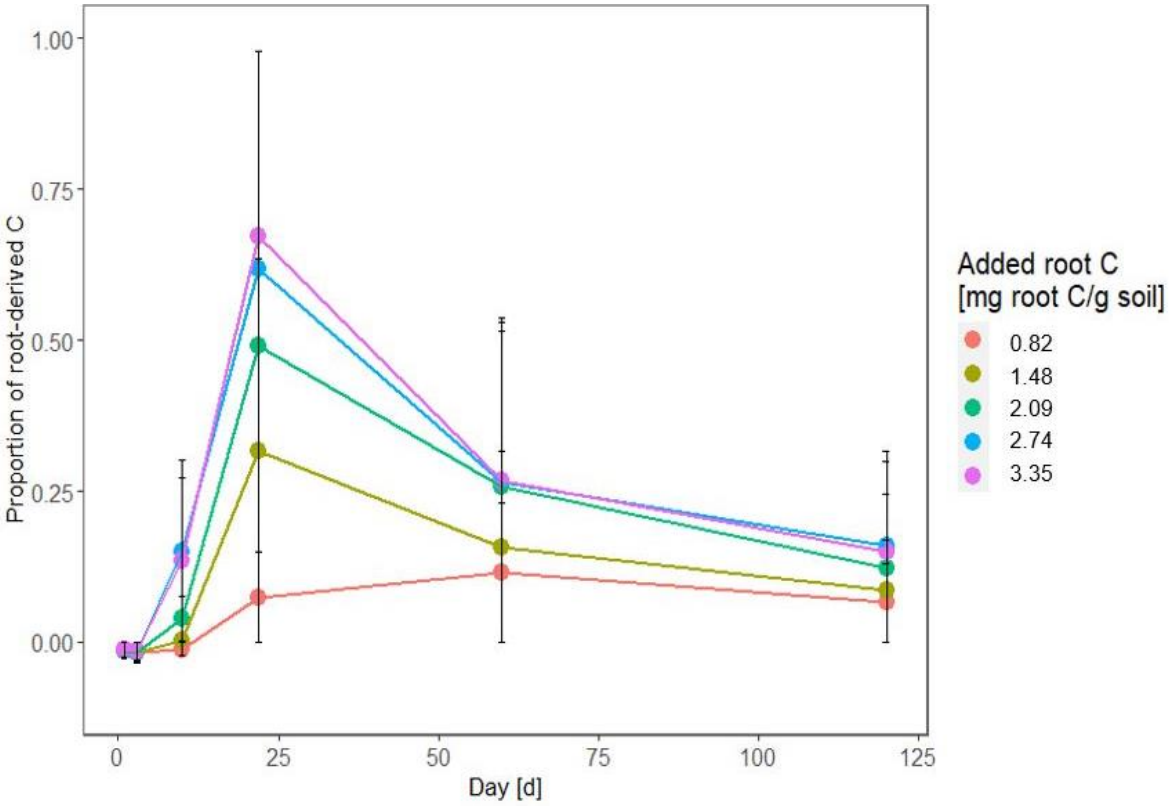

Figure A3. Proportion of root-derived CH_4-C over time in the saturated treatments. Again, the system does not need much time to stabilize. The increase of the proportion reaches its peak already at day 22 and drops rapidly thereafter. It is also remarkable that a significantly higher proportion of CH_4-C comes from the roots than CO_2-C (Figure A3).

8.3 CH4-C as CO2-C equivalent

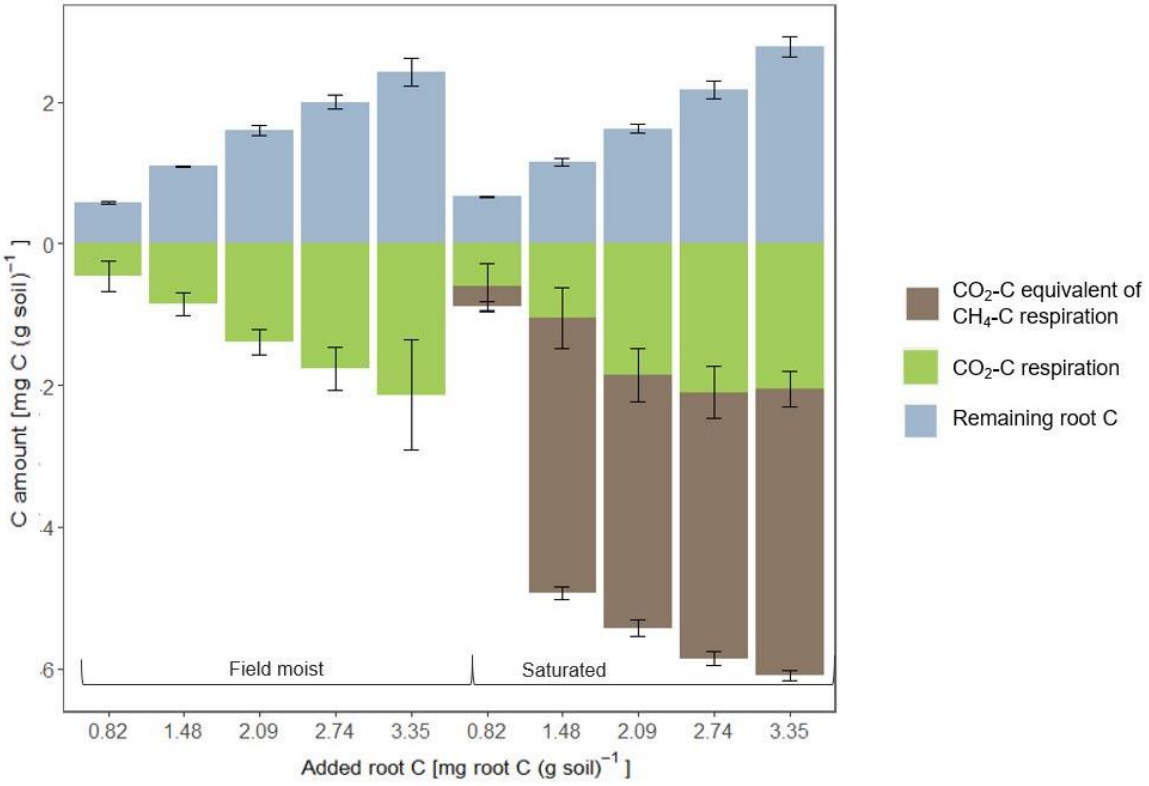

Figure A4. Converting the CH_4-C to its CO_2-C equivalent of the heat-binding capacity. The calculation was simply by multiplying the CH_4-C values in [mg] with the factor 25 which is the heat-binding capacity of CH_4 compared to CO_2 (Bridgham et al., 2013; Forster et al., 2007). Comparing this figure with Figure 7, the CH_4-C converted to CO_2-C has a much larger impact on ecosystems.

8.4 Treatment list

Table A1. Data and setup of treatments before incubation.

ID	Treatment	Replication	Wet soil mass [mg]	Added fine root mass [mg]	Added water [ml]
1	1	1	64045.2	70.33	6.4
2	1	2	63824.5	69.93	6.4
3	1	3	64176.9	70.64	6.4
4	1	4	63537.7	70.38	6.4
5	2	1	64188.5	122.71	6.4
6	2	2	63890.4	122.14	6.4
7	2	3	64053.7	121.26	6.4
8	2	4	64514.4	121.77	6.4
9	3	1	64266.1	174.24	6.4
10	3	2	64334.6	173.87	6.4
11	3	3	64713.5	175.09	6.4
12	3	4	64370.12	174.28	6.4
13	4	1	64285.65	226.2	6.4
14	4	2	64362.95	226.82	6.4
15	4	3	64304.95	227.01	6.4
16	4	4	64250.8	227.26	6.4
17	5	1	64178.35	279.7	6.4
18	5	2	64072.47	279.19	6.4
19	5	3	64176.42	279.26	6.4
20	5	4	64095.6	279.02	6.4
21	6	1	64480.9	70.62	156.4
22	6	2	64044.8	69.9	156.4
23	6	3	64216.7	69.51	156.4
24	6	4	64104.15	70.08	156.4
25	7	1	64524.34	122.35	156.4
26	7	2	64333.9	121.96	156.4
27	7	3	64251.5	121.62	156.4
28	7	4	64414.46	122.02	156.4
29	8	1	64391.7	174.16	156.4
30	8	2	64102.3	174.49	156.4
31	8	3	64026.75	173.22	156.4
32	8	4	64009.8	175.1	156.4
33	9	1	64344.7	225.56	156.4
34	9	2	64481.7	226.11	156.4
35	9	3	64376.27	225.9	156.4
36	9	4	64019.8	225.74	156.4
37	10	1	64529.17	279.34	156.4
38	10	2	64565.3	277.46	156.4
39	10	3	64246.5	280.22	156.4
40	10	4	64152.58	278.55	156.4
41	11	1	64243.8	0	6.4

42	11	2	64252.3	0	6.4
43	11	3	648095.6	0	6.4
44	11	4	64017.2	0	6.4
45	12	1	64477.6	0	156.4
46	12	2	64283.1	0	156.4
47	12	3	64531.1	0	156.4
48	12	4	64252.7	0	156.4
49	13	1	0	0	0
50	14	1	0	0	156.4
51	15	1	64619.17	279.6	0
52	16	2	64031.28	278.65	156.4

8.5 Personal declaration

I hereby declare that the submitted thesis is the result of my own, independent work. All external sources are explicitly acknowledged in the thesis.

Olten; June 30, 2023