

1 **Slicing a peat pie: just 5 cm of topsoil removal strongly reduces CH₄ emission after rewetting**

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14 **Abstract**

15 Topsoil removal (TSR) is a management action performed before rewetting drained agricultural peatlands to reduce
16 greenhouse gas (GHG) emissions and nutrient leaching. The current common practice to remove 30 to 60 cm of
17 topsoil is labor-intensive, costly, and highly invasive. However, the optimal TSR depth for mitigating carbon
18 emissions from rewetted peat soils has neither been determined nor linked to soil biogeochemical factors driving
19 carbon emissions.

20 We executed two mesocosm experiments to address this. In experiment 1, we removed the topsoil of two contrasting
21 peat soils before rewetting (i.e., extensively managed, acid peat *and* intensively managed, near-neutral peat) with
22 increments of 5 cm up to 25 cm TSR. In experiment 2, we combined TSR with the presence and absence of *Typha*
23 *latifolia* on intensively managed, near-neutral peat soil. The experiments ran for 22 and three months, respectively,
24 in which we measured carbon dioxide (CO₂) and methane (CH₄) emissions and porewater chemistry.

25 Our experiments show that i) 5 cm TSR greatly reduced CH₄ and CO₂ emissions irrespective of peat nutrient status
26 during the 22-month experiment, and ii) the presence of *T. latifolia* further reduced CH₄ emissions during the 3-
27 month experiment. Specifically, CH₄ emissions were six to 10-times lower with 5 cm TSR compared to 0 cm TSR.
28 Peak CH₄ emissions occurred after three months with 0 cm TSR and strongly decreased thereafter. Random forest
29 analyses showed that variation in CH₄ emissions could mainly be explained by cumulative root biomass and
30 porewater alkalinity. Furthermore, 5 cm TSR reduced porewater values of pH, alkalinity, CH₄, and ammonium. The
31 effectiveness of TSR in preventing the build-up of phosphorus, iron, and sulfur in porewater was site-specific.

32 This experimental study highlights that only 5 to 10 cm TSR may already effectively prevent adverse effects of
33 peatland rewetting by cutting undesirable CH₄ emissions and avoiding nutrient release. This study clarifies that
34 target settings and site-specific assessments are crucial to decide on the amount of TSR for peatland restoration and
35 paludiculture.

36 **Keywords:** greenhouse gas emissions, peatland restoration, climate mitigation, root biomass, TSR, *Typha*

37 **1 Introduction**

38 Although peatlands cover only 3% of the global land area, they store 30% of global soil carbon (Yu 2012; Leifeld
39 and Menichetti 2018; Chaudhary et al. 2020). Historical drainage for agriculture has turned large areas of peatlands
40 into carbon sources, accounting for 5% of the global anthropogenic CO₂ emissions (Cobb et al. 2017; Chaudhary et
41 al. 2020; IPCC 2021). To mitigate carbon emissions from peatlands and restore their function as carbon sinks
42 worldwide, peatland rewetting is proposed as an important measure (IPCC 2021; Evans et al. 2021; Temmink et al.
43 2022). While increasing water levels in drained peatlands can reduce CO₂ emissions, they are known to stimulate
44 CH₄ emissions (Evans et al. 2021). In the long-term, however, rewetting degraded peat benefits the climate despite
45 CH₄ emissions (Günther et al. 2020).

46 In natural peat soils, a high-water table leads to anoxic conditions due to continued oxygen (O₂) consumption and
47 near-absent O₂ intrusion (Laanbroek 1990; Rydin and Jeglum 2013; Conrad 2020). Rewetting of peat soils results in
48 rapid depletion of O₂ availability and initiates the reduction of alternative electron acceptors, preferentially in the
49 sequence of nitrate, manganese, ferric iron, and sulfate before CH₄ production starts (Glaser and Chanton 2009;
50 Rydin and Jeglum 2013; Conrad 2020). CH₄ production is mainly substrate-limited (Segers 1998; Drake et al. 2009),
51 and in peatlands, pathways of CH₄ production are primarily the reduction of acetate (more than 70%) and CO₂
52 (Smolders et al. 2002; Artz 2009). Therefore, CH₄ production is suppressed as long as concentrations of
53 methanogenic substrates (e.g., H₂, acetate) are limited by the presence of alternative electron acceptors (Conrad
54 2020). The availability of alternative electron acceptors and the time required for the growth of methanogenic
55 archaea and other anaerobic microorganisms result in a lag phase of CH₄ production (Hahn-Schöfl et al. 2011;
56 Conrad 2020). Rewetting may thus shift drainage-based CO₂-emission to CH₄-dominated emissions (Wilson et al.
57 2016a; Renou-Wilson et al. 2019; McNicol et al. 2020).

58 Topsoil removal (TSR) has recently been shown as an effective measure to reduce GHG emissions after peatland
59 rewetting on a field scale (Harpenslager et al. 2015; Huth et al. 2020). The main mechanism of this reduction by soil
60 preparation is the removal of the peat layer with the highest labile carbon content, including shoots, roots, and humic
61 soil (Harpenslager et al. 2015; Huth et al. 2020). The standard practice of TSR is to remove about 30 to 60 cm of
62 peat (Allison and Ausden 2004; Gaudig et al. 2018; Huth et al. 2020). This depth is often used to remove labile
63 carbon and the nutrient-rich topsoil layer, which is especially important for paludiculture practices (i.e., the

64 cultivation of crops on wet or rewetted agricultural peatlands). Since rewetted peatlands are often former agricultural
65 sites, the maintained productivity of a site is often preferred, as it is a win-win situation for the climate and the
66 landowner (Budiman et al. 2020). For instance, in *Sphagnum* paludiculture, TSR minimizes the competition between
67 mosses and fast-growing plants (Gaudig et al. 2017), but also removes the highly decomposed layer with low
68 porosity and hydraulic conductivity that hampers *Sphagnum* growth (Gaudig et al. 2018; Grobe et al. 2021). Besides
69 causing a substantial change in the environment, TSR is a costly practice and may thus put a large constraint on the
70 willingness to restore peatland functioning by rewetting (Klimkowska et al. 2010; Zak et al. 2018; Convention on
71 Wetlands 2021).

72 Interestingly, the mechanisms driving the contribution of individual soil layers and vegetation of rewetted peat to
73 GHG emissions are poorly understood. For instance, the factors ruling hotspot formation of CH₄ production,
74 including electron acceptor availability and shunt effects of tall growing plant species, are not fully known (Fenner
75 et al. 2011). Although CH₄ production is expected to occur after the depletion of alternative electron acceptors
76 (Smolders et al. 2002; Hahn-Schöfl et al. 2011; De Jong et al. 2020), researchers also found contrasting results
77 (Knorr and Blodau 2009). Similarly, the presence of vascular plants can affect CH₄ emissions positively or
78 negatively by increasing CH₄ oxidation or favoring CH₄ transportation to the atmosphere by the roots (Fritz et al.
79 2011; Agethen et al. 2018; Huth et al. 2020).

80 To bring more clarity, we performed two mesocosm experiments, removing topsoil with 5-cm increments up to 25
81 cm before rewetting on nutrient-wise contrasting peat soils, and with and without the growth of *Typha latifolia*, a
82 common paludiculture species. We determined GHG fluxes and porewater chemistry during 22 and three months for
83 the first and second experiments, respectively. With this study, we aimed to answer the following research
84 questions:

- 85 1. What is the mitigation potential of minimal TSR (i.e., compared to the standard practice) and the presence
86 of *T. latifolia* on the GHG emission of rewetted peat soils?
- 87 2. How is the nutrient availability of rewetted peat soils affected by minimal TSR and the presence of *T.*
88 *latifolia*?
- 89 3. What are the main drivers of CH₄ emissions from rewetted peat soils?

90 Our hypotheses are: (1) we expect higher CH₄ emission in the 0 cm TSR treatment due to the higher availability of
91 labile carbon and nutrients. In addition, we expect higher CH₄ emissions in the presence of *T. latifolia* due to the
92 increased transportation of CH₄ from the soil to the atmosphere (shunt effect). (2) We expect reduced nutrient
93 concentrations with TSR due to direct nutrient removal. In addition, we expect lower nutrient concentrations with
94 the presence of *T. latifolia* due to plant uptake. (3) We expect the labile carbon to be the biggest driver of CH₄
95 emissions from rewetted peat soils by limiting or supporting methanogenesis.

96 **2 Materials and methods**

97 *2.1 Sampling*

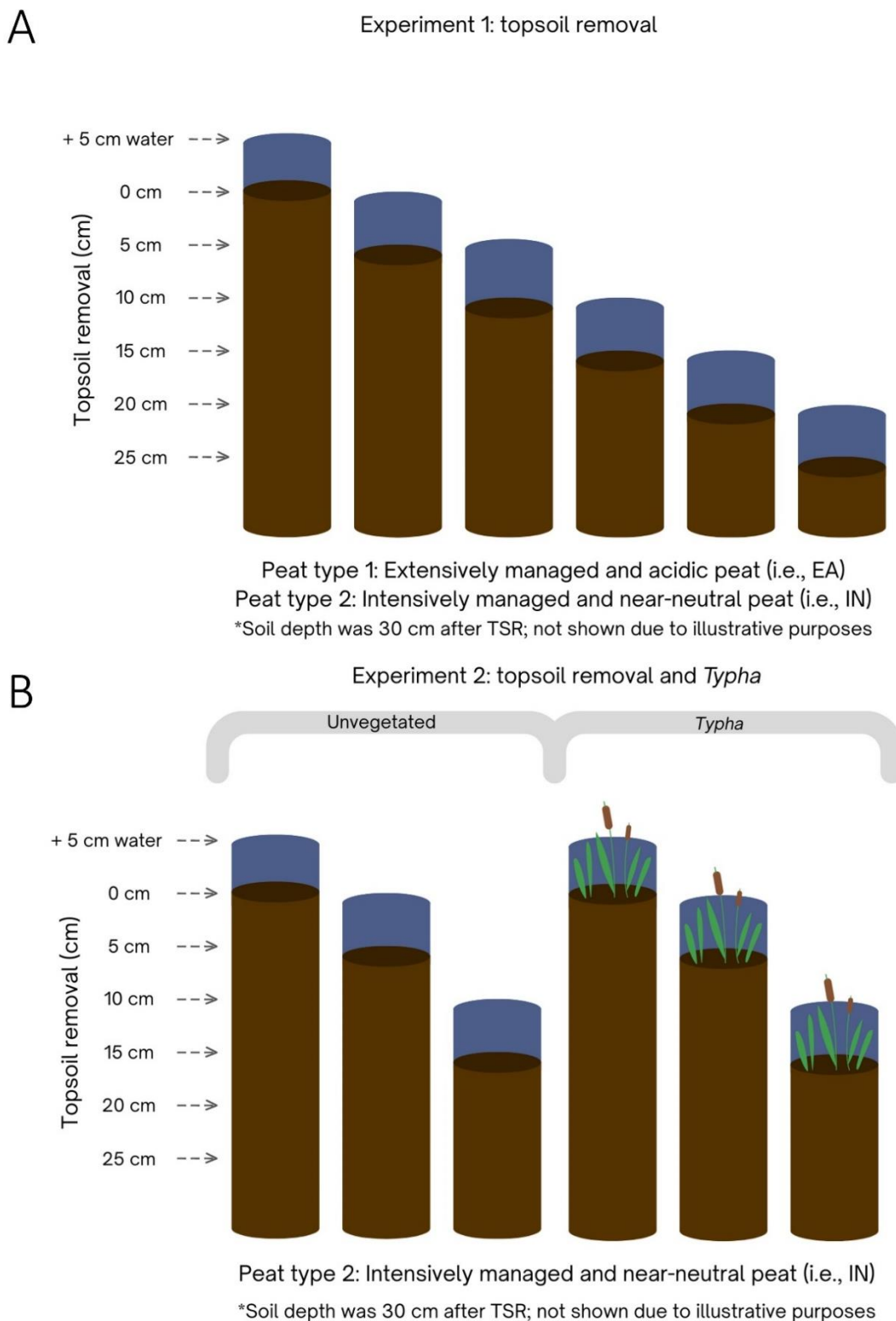
98 At two sites in the Netherlands, peat soil cores with a depth of 30 cm were taken using 15 cm diameter PVC-pipes at
99 two sites (Vroom et al. 2022). The first site is an extensively managed agricultural peat meadow in Bûtefjild
100 (coordinates: 53°15'N, 5°57'E), which has acid peat (pH 4.4), and it will be referred to as EA (i.e., extensively
101 managed and acid peat). The second site is a drained, intensively managed agricultural peat meadow in Zegveld
102 (coordinates: 52°08'N, 4°50'E), which has near-neutral peat (pH 5.6), and it will be referred to as IN (i.e., intensively
103 managed and near-neutral peat). We collected these contrasting sites to assess the site and history dependency on
104 GHG fluxes and nutrient removal (i.e., different agricultural history, nutrient availability, and pH). These cores were
105 used for two separate experiments. For experiment 1, both peat types were investigated, while for experiment 2, only
106 IN was used.

107 *2.2 Experiment 1: topsoil removal every 5 cm*

108 In the first experiment, we investigated the effects of TSR on GHG emissions and nutrient availability by removing
109 the topsoil of the collected peat cores at 5 cm intervals, incrementally, up to 25 cm (Fig. 1A). In the 0 cm TSR, only
110 the aboveground grass biomass was removed, leaving 5 cm long stubbles. The experiment was performed using four
111 replicates distributed randomly in a dark climate chamber of the Radboud University in the Netherlands with an air
112 temperature of 15 °C and relative humidity of 70%. The peat cores were placed in slightly larger cylinders and
113 inundated with 5 cm of demineralized water. The water level was kept constant throughout the experiment by the
114 regular addition of demineralized water. The experiment ran for 22 months, from March 2016 until December 2017.

115 2.3 Experiment 2: interaction between topsoil removal and presence of *T. latifolia*

116 In the second experiment, we investigated the effects of TSR coupled with the presence of *T. latifolia*, a typical
117 paludiculture and wetland species, on CH₄ emissions and nutrient availability. Only IN soil was used, with three
118 TSR depths (0, 5, and 15 cm) in the presence and absence of *T. latifolia* (Fig. 1B). In contrast to experiment 1, in the
119 0 cm TSR the aboveground grass biomass was not trimmed back. The cores were placed in a temperature-controlled
120 water bath at 14 °C in the Radboud University greenhouse facilities after they were closed at the bottom with a PVC
121 cap and were placed in a plastic bag (open at the top). Each core was inundated up to the peat surface using
122 rainwater. For each TSR treatment, five cores were planted with three *T. latifolia* seedlings (\pm 5 cm each) (on day 0;
123 total $n = 15$). Seedlings were raised in the greenhouse from seeds collected at Deurnese Peel in the Netherlands
124 (coordinates: 5°52'29''E; 51°25'20''N). Three cores per TSR treatment were not vegetated and served as controls
125 (total $n = 9$). The unvegetated cores were covered by dark perforated plastics to avoid algae growth. For brevity, the
126 treatments with *T. latifolia* are called 'Typha' and unvegetated controls 'unvegetated' hereafter. Twelve days after
127 planting the seedlings, the water table in all cores was raised to 5 cm above the peat surface and kept steady
128 throughout the experiment by the regular addition of rainwater. The experiment ran for three months, from April to
129 June 2018.



130

131 **Fig. 1** Overview of the experimental setup of experiments 1 (A) and 2 (B). Soil depth was 30 cm after topsoil

132 removal (TSR); not shown due to illustrative purposes.

133 *2.4 Measurements*

134 Methane (CH₄) and carbon dioxide (CO₂) fluxes were measured six times during experiment 1 (months 1, 2, 3, 10,
 135 19, and 22) and three times during experiment 2 (months 1, 2, and 3). Gas measurements were carried out using a
 136 dark PVC chamber (15 cm inner diameter), which was connected to a Los Gatos Greenhouse Gas Analyzer (GGA-
 137 24EP, Los Gatos Research, Mountain View, CA, USA) for experiment 1 and to an Ultraportable Greenhouse Gas
 138 Analyzer (UGGA-30EP, Los Gatos Research, Mountain View, CA, USA) for experiment 2. A battery-driven fan
 139 allowed constant airflow in the chamber. The measurements lasted for 180 seconds and were repeated if ebullition
 140 was observed (i.e., in case of an abrupt increase in gas concentration). Simultaneously to the gas measurements, the
 141 air temperature was logged using a HOBO logger (Onset Computer Corporation, Bourne, MA, USA). CH₄ and CO₂
 142 fluxes (mg m⁻² d⁻¹) were calculated following Almeida et al. (2016):

$$143 \quad F = \frac{V}{A} * slope * \frac{P * F1 * F2}{R * T}$$

144 Where F is CH₄ or CO₂ flux (mg m⁻² d⁻¹); V is chamber volume (m³); A is chamber surface area (m²); slope is the
 145 slope of the relationship between CH₄ or CO₂ and time (ppm s⁻¹); P is atmospheric pressure (kPa); F1 is the
 146 molecular weight (CO₂: 44 and CH₄: 16 g mole⁻¹); F2 is the conversion factor of seconds to days; R is the gas
 147 constant (8.3144 J K⁻¹ mole⁻¹); and T is the temperature in Kelvin (K).

148 CH₄ in the porewater from the upper 10 cm of peat was sampled twice during experiment 1 (months 2 and 3), and
 149 three times during experiment 2 (months 1, 2, and 3). The porewater CH₄ was collected using rhizon samplers
 150 (Rhizosphere Research Products, Wageningen, The Netherlands) attached with a needle to a pre-vacuumed 12 mL
 151 exetainer (Labco, Lampeter, UK) containing 1 mL of hydrochloric acid (0.1 M) to stop the biological activity. The
 152 samples were, then, analysed by equilibrating with the headspace of ambient air immediately after sampling and
 153 measuring the gas phase on an HP 5890 gas chromatograph equipped with a Porapak Q column (80/100 mesh) and a
 154 flame ionization detector (GC-FID, Hewlett Packard, Palo Alto, CA, USA). Concentrations were calculated using
 155 Henry's law (Sander 2015).

156 Porewater chemical composition from the upper 10 cm of peat was determined from samples taken four times
 157 during experiment 1 (months 1, 2, 3, and 10) and three times during experiment 2 (months 1, 2, and 3) using the

158 same rhizon samplers attached to a syringe under vacuum. The samples were analysed following the approach
159 described by Vroom et al. (2022) to determine pH, alkalinity, ammonium (NH_4^+), phosphorus (P), iron (Fe), and
160 sulfur (S). The pH and alkalinity were determined using Ag/AgCl electrode (Orion Research, Beverly, MA, USA)
161 and a TIM 840 Titration Manager (Radiometer Analytical SAS, Villeurbanne, France). NH_4^+ was determined by
162 colorimetric methods (Auto Analyser III, Bran and Luebbe GmbH, Norderstedt, Germany). P, Fe, and S were
163 determined using an inductively coupled plasma emission spectrometry (ICP-OES, IRIS Intrepid II, Thermo
164 Electron Corporation, Franklin, MA, USA).

165 For experiment 1, root samples were washed from additional cores that were not rewetted ($n = 4$). The 15 cm
166 diameter cores were sliced every 5 cm to a depth of 30 cm. The samples were thoroughly rinsed to retain the roots
167 (living and dead), after which the material was weighed, dried at 70 °C for 96 hours, and weighed again.

168 2.5 Statistical analyses

169 GHG emissions were log-transformed before all analyses. To enable the inclusion of extremely low CH_4 fluxes (± 1
170 $\text{mg m}^{-2} \text{day}^{-1}$), these were set to 0 after the transformation. We did not filter fluxes based on their fit (R^2). Negative
171 fluxes ($< -1 \text{ mg m}^{-2} \text{day}^{-1}$; $n = 7$ for CH_4 from experiment 1, $n = 0$ for CH_4 from experiment 2, and $n = 15$ for CO_2
172 from experiment 2) were removed from all analyses, since this study focused on emissions only.

173 The effect of TSR depths and the effect of *Typha* on GHG emissions (RQ1) of rewetted peat soils were assessed
174 using three-way repeated-measures ANOVAs. For experiment 1, differences in CH_4 emission and CO_2 emission
175 were separately assessed using TSR treatment, peat type (EA vs. IN), time (the month into the experiment), and their
176 interactions as independent variables. For experiment 2, differences in CH_4 emission were assessed using TSR
177 treatment, plant presence, time (the month into the experiment), and their interactions as independent variables, after
178 extreme outliers' removal ($n = 1$). Since the response variables were not normally distributed according to the
179 Shapiro-Wilk normality test, differences between individual treatments that proved significant in the ANOVA were
180 tested using the non-parametric Wilcoxon paired samples post-hoc tests with Bonferroni correction of p values.

181 The effect of TSR depths and the effect of *Typha* growth on nutrient availability (RQ2) of rewetted peat soils were
182 assessed using three-way repeated-measures ANOVAs. For experiment 1, differences in the grass and root biomass,
183 porewater CH_4 concentrations, and porewater nutrient concentrations were separately assessed using TSR treatment,

184 peat type (EA vs. IN), time (the month into the experiment), and their interactions as independent variables. For
185 experiment 2, differences in porewater CH₄ concentrations and nutrient concentrations were assessed using TSR
186 treatment, plant presence, time (the month into the experiment), and their interactions as independent variables. To
187 test for differences between individual treatments that proved significant in the ANOVA, we used pairwise t-tests
188 for porewater alkalinity, pH, P, and Fe and the non-parametric Wilcox paired samples test for porewater CH₄, NH₄⁺,
189 and S because these variables were not normally distributed. All post-hoc tests were performed with Bonferroni
190 correction of *p* values.

191 We performed two random forest analyses for both experiments to determine the main drivers of the mitigation
192 potential for CH₄ emissions (RQ3). For experiment 1, measurements over months 1, 3, and 10 were used as they had
193 complete measurements. The CH₄ fluxes were the dependent variable, and CO₂ flux, cumulative CO₂, cumulative
194 root biomass, time (the month into the experiment), soil type, and porewater results (alkalinity, pH, NH₄⁺, Fe, S, and
195 P) were used as independent variables. For experiment 2, CH₄ emissions were also used as the dependent variable,
196 and time (the month into the experiment), vegetation treatment, and porewater results (alkalinity, pH, NH₄⁺, Fe, S,
197 and P) as independent variables. Porewater CH₄ concentration was not included in the random forest, because it was
198 not measured during the whole experiment 1 due to logistical constraints. Therefore, the relation between porewater
199 CH₄ concentrations and CH₄ fluxes were tested separately using linear regression.

200 The statistical analyses were conducted in R (R core team, 2016), where the random forest analysis was performed
201 using the ‘RandomForest’ package (Liaw and Wiener 2002). Graphs were created using JPM (JMP 2021). All data
202 are shown with their average ± standard deviation.

203 **3 Results**

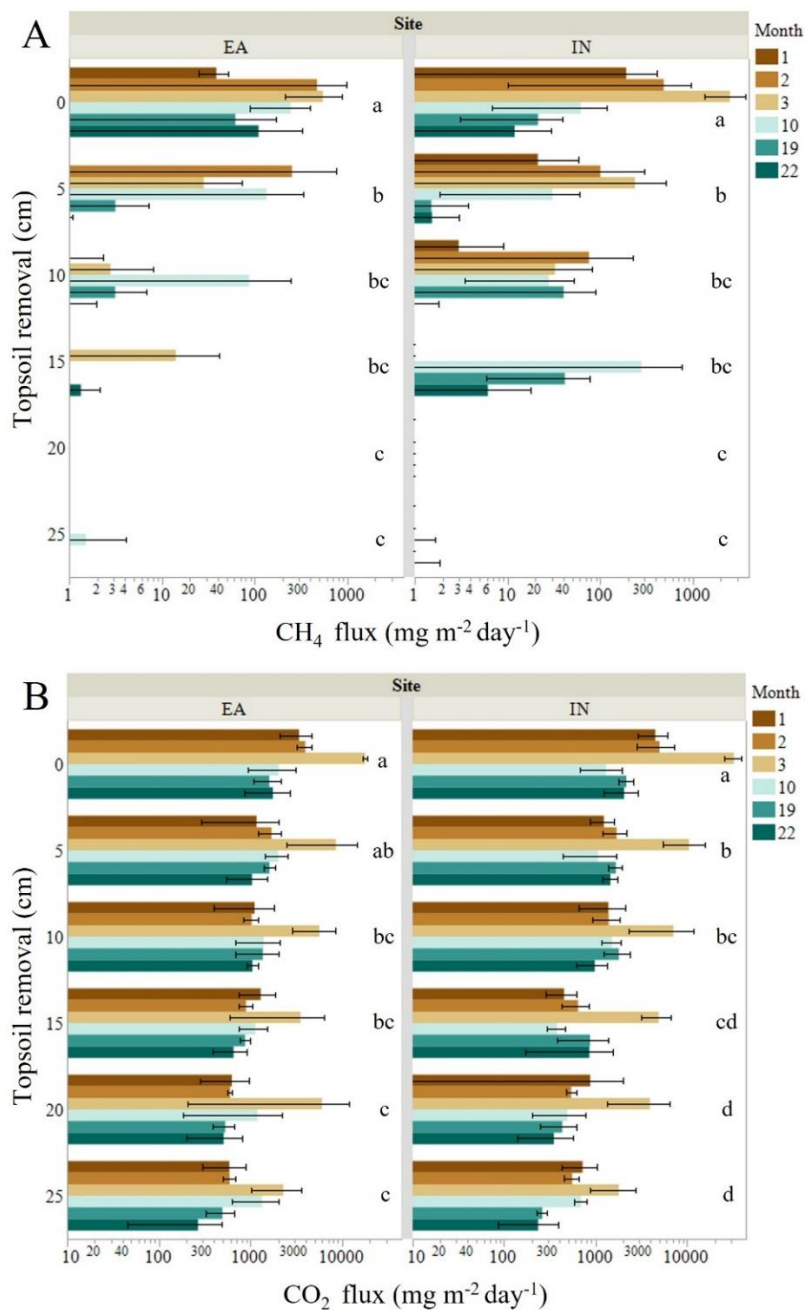
204 *3.1 RQ1: mitigation potential of TSR and the presence of Typha on the GHG emission*

205 *3.1.1 Experiment 1: topsoil removal every 5 cm*

206 CH₄ emissions differed over time and with different amounts of TSR, but not between peat types (Fig. 2A; Table S1
207 and Table S2). The highest CH₄ emissions were found in the 0 cm TSR treatment (overall average ± standard
208 deviation: 395 ± 758 mg CH₄ m⁻² day⁻¹) and were already 6-times lower with TSR of 5 cm (67 ± 186 mg CH₄ m⁻²

209 day⁻¹). CH₄ emissions further decreased to 23 ± 67 mg CH₄ m⁻² day⁻¹ at 10 cm TSR, 23 ± 122 mg CH₄ m⁻² day⁻¹ at
210 15 cm TSR, and less than 1 mg CH₄ m⁻² day⁻¹ at 20 and 25 cm TSR (Fig. 2A). Emissions peaked after three months
211 for the 0, 5, and 10 cm TSR and gradually declined afterward (Fig. 2A).

212 CO₂ emissions differed between TSR treatments and over time, but not between peat types (Fig. 2B; Table S3). The
213 highest CO₂ emissions were found in the treatment with 0 cm TSR (overall average ± standard deviation: 6496 ±
214 9243 mg CO₂ m⁻² day⁻¹). CO₂ emissions decreased with an increase in TSR: 2655 ± 3798 mg CO₂ m⁻² day⁻¹ with 5
215 cm, 1967 ± 2468 mg CO₂ m⁻² day⁻¹ with 10 cm, 1309 ± 1685 mg CO₂ m⁻² day⁻¹ with 15 cm, 1423 ± 2469 mg CO₂ m⁻²
216 day⁻¹ with 20 cm, and 617 ± 885 mg CO₂ m⁻² day⁻¹ with 25 cm. Despite water-logged conditions, TSR treatments
217 caused CO₂ emissions to differ by a factor of 10.

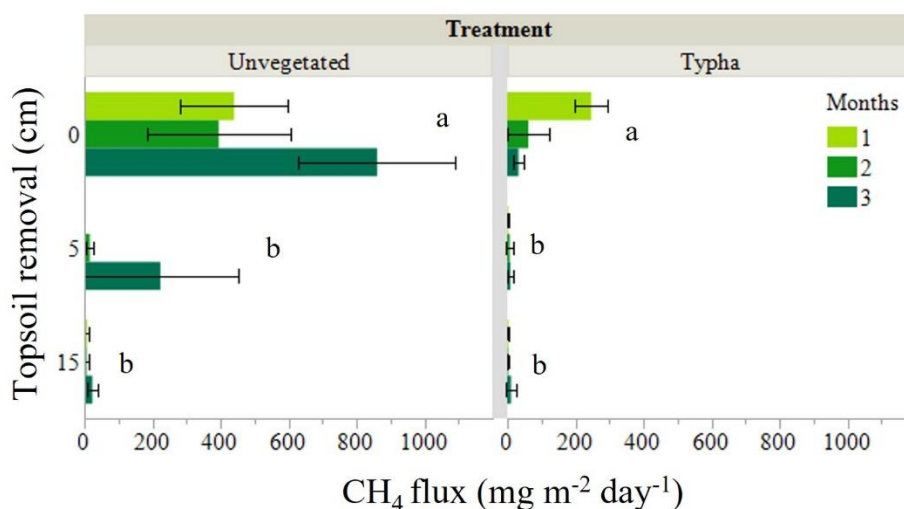


218

219 **Fig. 2** Emissions of (A) methane (CH₄; mg m⁻² day⁻¹) and (B) carbon dioxide (CO₂; mg m⁻² day⁻¹) under water-
 220 logged conditions per topsoil removal increment (cm) over time and per peat type. EA = extensively managed, acid
 221 peat. IN = intensively managed, near-neutral peat. Note: the x-axes are presented on a logarithmic scale. Bars
 222 represent the average and standard deviation (*n* = 4). Colors indicate the time of measurement (months into the
 223 experiment). Letters indicate significant differences between TSR depth per peat type (*p* < 0.05). Data from
 224 experiment 1.

225 3.1.2 Experiment 2: interaction between topsoil removal and presence of *T. latifolia*

226 CH₄ emissions differed over time, with different amounts of TSR and with the presence or absence of *Typha* (Fig. 3;
 227 Table S4 and Table S5). The lowering effect of TSR on CH₄ emissions remained when *Typha* was present.
 228 Vegetated mesocosms in combination with TSR revealed the lowest CH₄ emissions (Fig. 3). In the presence of
 229 *Typha*, 5 cm TSR significantly reduced CH₄ emissions by ~20 times, from 115 ± 113 mg CH₄ m⁻² day⁻¹ at 0 cm TSR
 230 to 6 ± 10 mg CH₄ m⁻² day⁻¹ at 5 cm TSR (Fig. 3; $p < 0.001$). In unvegetated treatments, 5 cm TSR significantly
 231 reduced CH₄ emissions by more than 8 times, from 523 ± 276 mg CH₄ m⁻² day⁻¹ at 0 cm TSR to 64 ± 137 mg CH₄
 232 m⁻² day⁻¹ at 5 cm TSR (Fig. 3; $p < 0.001$). There was no further significant decrease in emissions from 5 cm to 15 cm
 233 TSR. CH₄ emissions appeared to increase over time, except for mesocosms with *Typha* at 0 cm TSR where
 234 emissions appeared to decrease. However, none of these differences were significant (Table S4 and Table S5).

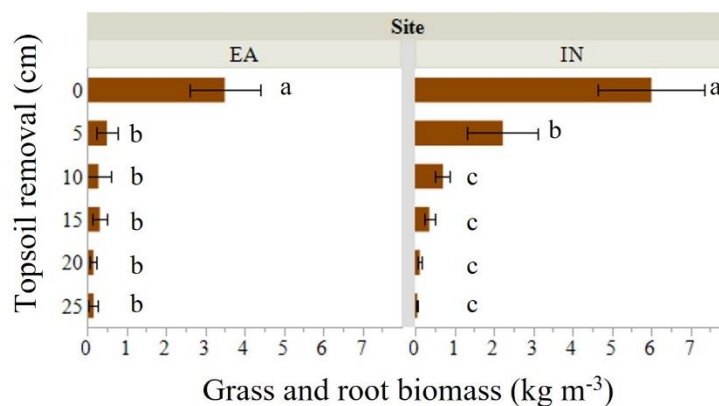


235
 236 **Fig. 3** Methane (CH₄) emissions (mg m⁻² day⁻¹) per topsoil removal increment (cm). Distinctions have been made
 237 over time (months into the experiment, indicated by color) and between treatments (i.e., unvegetated and *Typha*).
 238 Data from experiment 2, where only one peat type (IN) was used. Note: CH₄ fluxes are not shown on a logarithmic
 239 scale. Bars represent the average and standard deviation ($n = 5$ for *Typha*, $n = 3$ for unvegetated). Letters indicate
 240 significant differences between TSR depth per vegetation treatment ($p < 0.05$).

241 **3.2 RQ2: effects of TSR and the presence of *Typha* on nutrient availability**

242 **3.2.1 Experiment 1: topsoil removal every 5 cm**

243 Grass and root biomass, our proxy for easily decomposable carbon, differed per TSR treatment and per peat type (p
244 = 0.004; Fig. 4, Table S6). Overall, values were higher in the 0 cm TSR treatment and on IN peat (Fig. 4, Table S6).

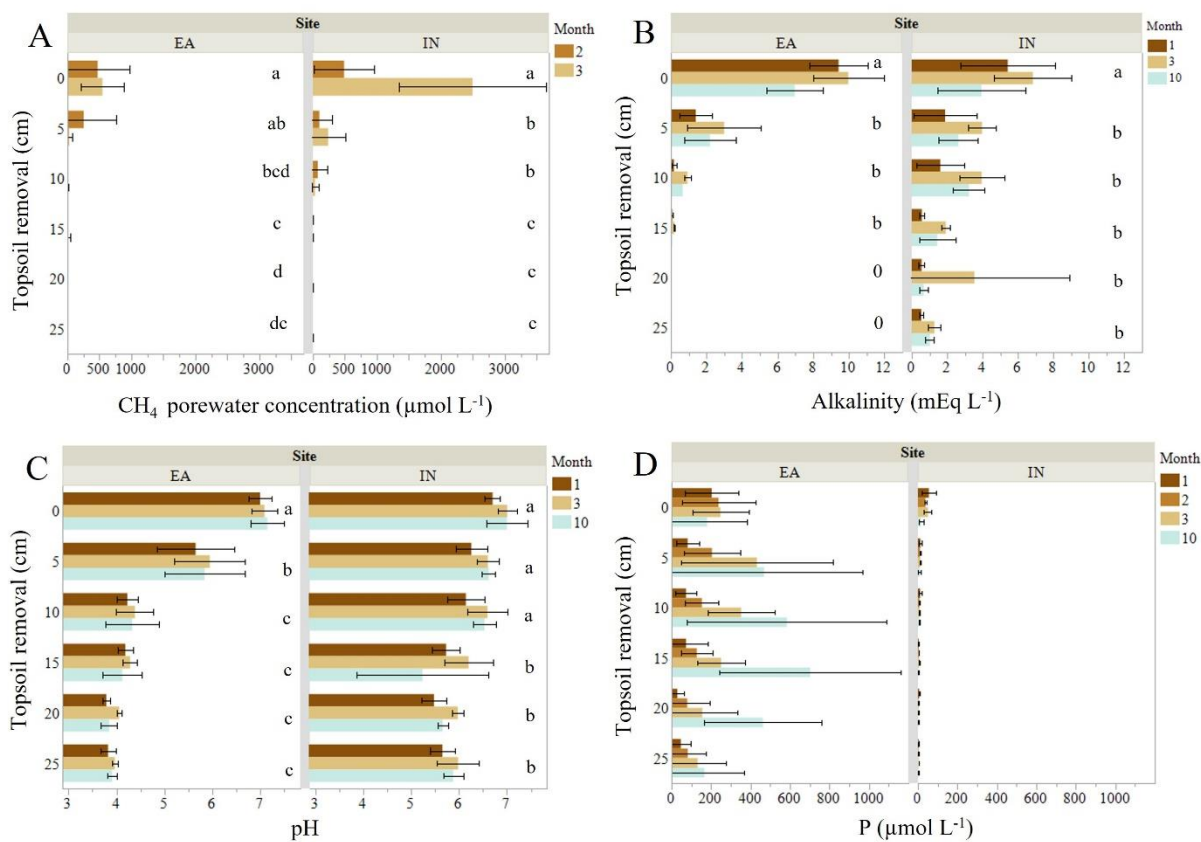


245
246 **Fig. 4** Grass and root biomass per volume (kg dry weight m⁻³) per topsoil removal increment (cm) in the extensively
247 managed, acid peat (EA) and in the intensively managed, near-neutral peat (IN). Bars represent the average and
248 standard deviation ($n = 4$). The 0 cm TSR treatment contained both aboveground biomass (leaf and shoot biomass)
249 and belowground biomass (grass roots). Letters indicate significant differences between TSR depth per peat type (p
250 < 0.05). Data from experiment 1.

251 Porewater CH₄, pH, alkalinity, P, and NH₄⁺ values differed per peat type, but not porewater Fe and S concentrations
252 (Fig. 5, Table S7, Fig. S1). TSR affected porewater values of CH₄, pH, alkalinity, NH₄⁺, Fe, and S, except for P
253 concentrations (Fig. 5, Table S7, Fig. S1). Moreover, porewater values of pH, alkalinity, P, NH₄⁺, Fe, and S changed
254 over time, except for CH₄ concentrations (Fig. 5, Table S7, Fig. S1).

255 CH₄ porewater concentrations declined with increasing TSR (Fig. 5A, Table S7). The highest CH₄ concentrations
256 were found in the treatments with 0 cm TSR (EA = 665 ± 500 μmol L⁻¹; IN = 1624 ± 1218 μmol L⁻¹). The 5 cm
257 TSR treatment lowered CH₄ porewater by 2 and 12-times (EA = 223 ± 312 μmol L⁻¹; IN = 135 ± 236 μmol L⁻¹). The
258 decrease in pH with increasing TSR was more pronounced on EA peat, which lines up with the alkalinity being

259 lower in EA at increased TSR compared to IN (Fig. 5B and C, Table S7). Both P and Fe concentrations accumulated
 260 over time, showing higher concentrations in month 10 (Fig. 5D, Fig. S1B, Table S7). Contrary to the other elements
 261 where concentrations decreased with increased TSR, the concentrations of S species (oxidized, reduced, and
 262 elemental sulfur) increased overall (Fig. S1C, Table S7).

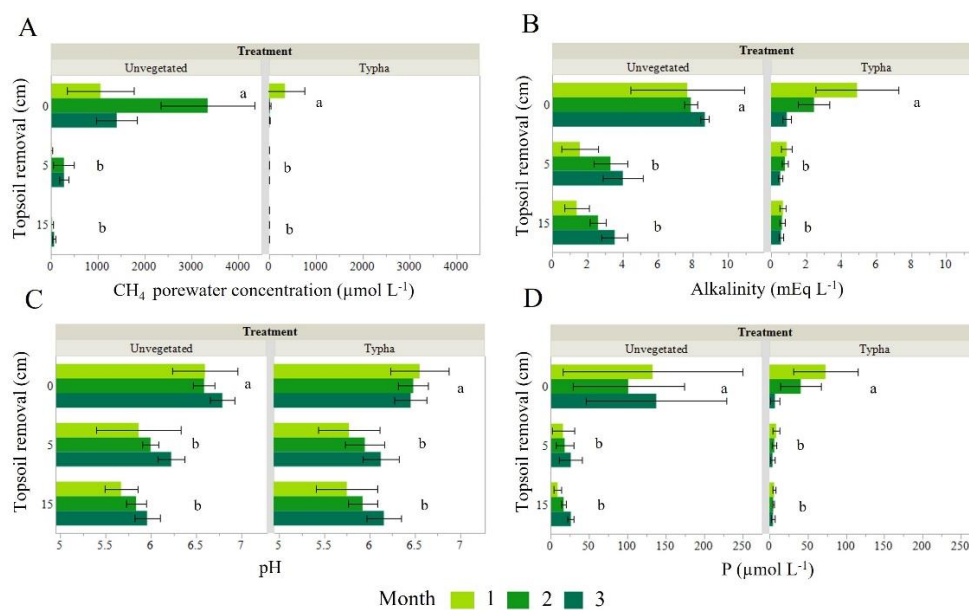


263

264 **Fig. 5** Porewater chemistry results of (A) methane (CH₄; μmol L⁻¹), (B) alkalinity (mEq L⁻¹), (C) pH, and (D)
 265 phosphorus (P; μmol L⁻¹) per topsoil removal increment (cm) over time and per peat type. EA = extensively
 266 managed, acid peat. IN = intensively managed, near-neutral peat. Bars represent the average and standard deviation
 267 ($n = 4$). Colors indicate the time of measurement (months into the experiment). Letters indicate significant
 268 differences between TSR depth per peat type ($p < 0.05$). Data from experiment 1.

269 3.2.2. Experiment 2: interaction between topsoil removal and presence of *T. latifolia*

270 Peat porewater chemistry was also affected by the presence of plants. Overall, porewater CH₄ concentrations were
 271 about 15 times lower in *Typha* treatments ($48 \pm 47 \mu\text{mol L}^{-1}$) than in unvegetated treatments ($729 \pm 386 \mu\text{mol L}^{-1}$; p
 272 < 0.001 ; Fig. 6A). CH₄ concentrations were higher in the 0 cm TSR treatments than 5 or 15 cm, with or without
 273 *Typha* (Fig. 6A). The addition of *Typha* and the different TSR treatments altered pH, alkalinity, P, NH₄⁺, and Fe
 274 values, but not S concentrations (Fig. 6, Fig. S2, Table S8). Only pH, NH₄⁺, Fe, and S values changed over time
 275 (Fig. 6, Fig. S2, Table S8). We observed decreased porewater NH₄⁺, P, and Fe concentrations in the *Typha*
 276 treatments (Fig. 6, Fig. S2, Table S8). The effect of TSR, independent of the presence of *Typha*, was observed for
 277 NH₄⁺ and P, with higher concentrations in the treatments with 0 cm TSR. In unvegetated treatments, TSR had a
 278 positive effect on Fe concentrations ($p < 0.001$) (Fig. S2B), while in both *Typha* and unvegetated treatments, S
 279 concentrations increased with increasing TSR ($p < 0.001$) (Fig. S2C).

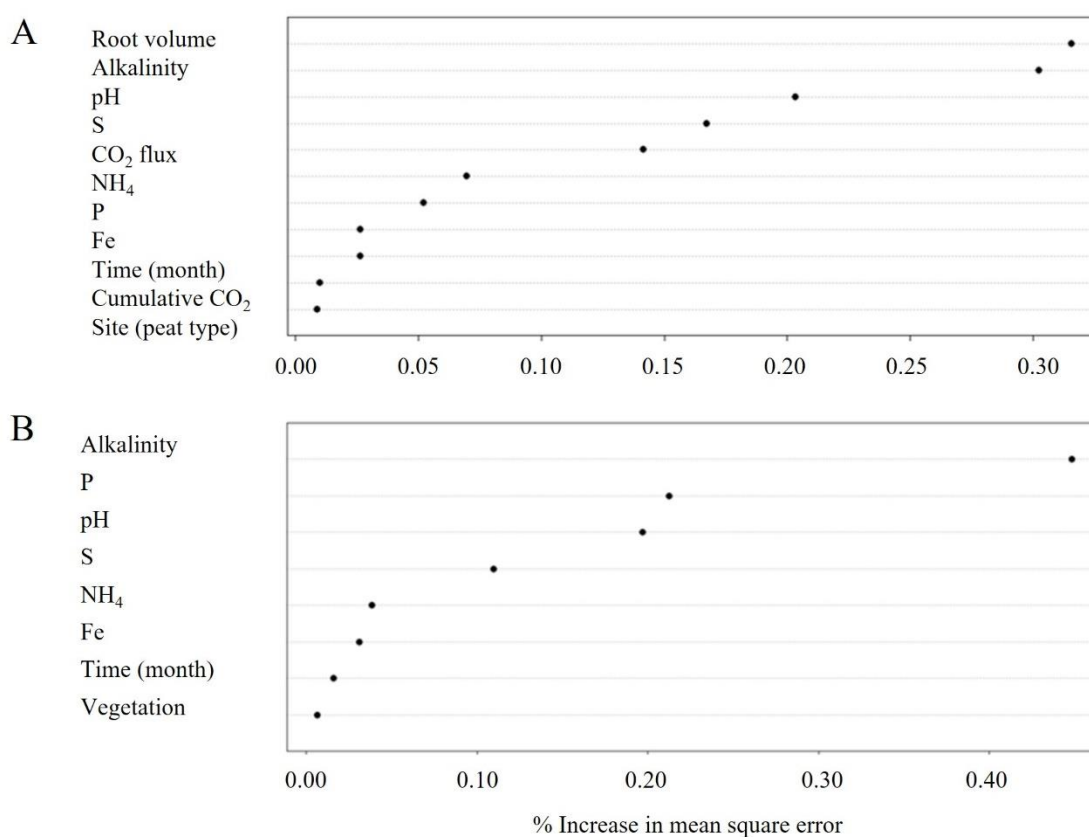


280

281 **Fig. 6** Porewater chemistry results of (A) methane concentration (CH₄; $\mu\text{mol L}^{-1}$), (B) alkalinity (mEq L^{-1}), (C) pH,
 282 and (D) phosphorus concentration (P; $\mu\text{mol L}^{-1}$) per topsoil removal increment (cm). Distinctions have been made
 283 over time (months into the experiment, indicated by color) and between treatments (i.e., unvegetated and *Typha*).
 284 Data from experiment 2, where only one peat type (IN) was used. Bars represent the average and standard deviation
 285 ($n = 5$ for *Typha*, $n = 3$ for unvegetated). Letters indicate significant differences between TSR depth per vegetation
 286 treatment ($p < 0.05$).

287 **3.3 RQ3: main drivers of CH₄ emissions**

288 For experiment 1, the random forest analysis showed that cumulative root biomass and porewater alkalinity were the
 289 most important determinants of CH₄ emissions (Fig. 7A). For experiment 2, porewater alkalinity and P
 290 concentration mostly explained CH₄ emissions (Fig. 7B). Furthermore, porewater CH₄ concentration showed a
 291 positive correlation with CH₄ flux for experiment 1 (Fig. S3; EA: $R^2 = 0.293$, $p < 0.0001$; IN: $R^2 = 0.402$, $p <$
 292 0.0001) and experiment 2 (Fig. S3; *Typha*: $R^2 = 0.64$, $p < 0.0001$; unvegetated: $R^2 = 0.16$, $p = 0.0157$).



293

294 **Fig. 7** Importance of different variables in predicting CH₄ emissions in the Random Forest analysis for experiment 1
 295 (A): MSE = 0.28, $R^2 = 0.75$, and experiment 2 (B): MSE = 11381, $R^2 = 0.75$. Cumulative root volume was not
 296 included for experiment 2 due to a lack of data. For experiment 1, we included measurements from months 1, 3, and
 297 10 only, since not all predictors were measured at other times.

298 **4 Discussion**

299 We showed that TSR has a strong effect on the availability of nutrients, (labile) carbon, and minerals, and the
300 consequent emission of CH₄ and CO₂ from rewetted agricultural peat. Specifically, the highest CH₄ emissions
301 occurred after three months with 0 cm TSR. Surprisingly, 5 cm TSR and 10 cm TSR largely reduced these high
302 GHG emissions post rewetting. Moreover, with increasing amounts of TSR, there was no additional significant
303 reduction in GHG emissions. Furthermore, climate benefits from shallow TSR prevailed in mesocosms planted with
304 *T. latifolia*, a wetland plant associated with high CH₄ emissions in wetlands. However, we stress that our vegetation
305 experiment only lasted for three months when roots potentially allow for minimal oxidation of submerged peat,
306 while root exudates and easily degradable plant litter of *Typha* can potentially boost CH₄ production and thus
307 emissions over time. Optimal TSR depth with the goal to reduce nutrient availability requires removal of at least 5
308 cm, while the inclusion of plants reduces the depth of peat that should be removed. Thus, as optimal TSR depth can
309 differ with the goal of rewetting the peatland, clear goal setting is vital to determine optimal TSR depth.
310 Nevertheless, our results suggest that the standard 30-60 cm of TSR is not always necessary to gain climate benefits
311 nor for nutrient reduction.

312 ***4.1 The mitigation potential of minimal TSR and Typha presence on GHG emissions***

313 We show that a mere 5 to 10 cm of TSR can sufficiently reduce CH₄ and CO₂ emissions from rewetted agricultural
314 peatlands. On average for the two peat types used in this study, we show that 5 cm of TSR reduces CH₄ emissions
315 by 1.9 tons of CH₄ ha⁻¹ yr⁻¹ and an additional 5 cm of TSR reduces emissions only further by 0.2 tons of CH₄ ha⁻¹ yr⁻¹
316 ¹. Although TSR is already known to reduce GHG emissions, previous studies applied at least 25 cm TSR
317 (Harpenslager et al. 2015; Zak et al. 2018; Huth et al. 2020). Therefore, our results suggest that the standard practice
318 of removing 30 to 60 cm of peat (Allison and Ausden 2004; Huth et al. 2020) is exceeding what would be required
319 from a climate change mitigation perspective. Rewetting alone reduced CO₂ emissions, which agrees with previous
320 studies (Wilson et al. 2016b; Günter et al. 2020; Huth et al. 2021). Important to consider that we did not include
321 controls without waterlogging, where CO₂ emissions would have been higher. However, the rewetting of peatlands
322 is found to favor CH₄ fluxes at the beginning (Wilson et al. 2009; Evans et al. 2021), while CH₄ emissions decrease
323 in the long-term (Günther et al. 2020). In this study, CH₄ emissions rose for three months followed by a marked
324 lowering in treatments where TSR was small or absent.

325 Besides the impact of TSR directly on the local environment and indirectly on costs, the removed peat needs to be
326 considered in the carbon balance of rewetted peat soils. Huth et al. (2021) found that TSR is a sustainable option for
327 climate mitigation even when the carbon losses in the removed topsoil were considered. However, minimizing
328 carbon export is critical and lines up with our findings that 5 cm TSR already represents a great gain in climate
329 mitigation.

330 Even though wetland vegetation is known to enhance CH₄ emissions by the shunt effect, transporting CH₄ from the
331 soil to the atmosphere (Fritz et al. 2011; Agethen et al. 2018; Huth et al. 2020), our data show that climate gains by
332 TSR are not offset by the presence of *Typha* in the first phase after rewetting. In our study, the presence of *Typha*
333 decreased CH₄ emissions to the atmosphere, most likely induced by rhizosphere oxidation. Similar results were
334 found in other short-term mesocosm experiments (Vroom et al. 2018) and for a broad range of wetland plants (Kao-
335 Kniffin et al. 2010). However, over time, higher CH₄ *in situ* emissions in peatlands with the presence of *T. latifolia*
336 are observed (Wilson et al. 2009). This suggests that *Typha* may be planted directly after rewetting but should be
337 harvested or removed before decay starts.

338 ***4.2 Effect of minimal TSR and Typha presence on nutrient availability***

339 In peatland restoration projects, TSR is also a promising practice to reduce internal eutrophication and pollution
340 downstream by reducing, for example, P mobilization (Allison and Ausden 2004; Harpenslager et al. 2015; Zak et
341 al. 2017; Zak et al. 2018). This reduction in nutrient availability can even be achieved with the minimal TSR
342 approach, as we found that porewater NH₄⁺ and Fe concentrations reduced significantly with 5 cm TSR. The
343 presence of *T. latifolia* further decreased the porewater concentrations of NH₄⁺ and Fe. Although we did not find a
344 clear reduction in porewater P concentration with minimal TSR, the presence of *T. latifolia* did lower P
345 mobilization. Peat type also had an influence on the effect of minimal TSR: near-neutral peat lowered P
346 mobilization compared to the more acid peat type, but Fe concentrations only reduced on near-neutral peat with
347 more than 15 cm TSR.

348 This suggests that, depending on peat pH, more or less TSR might be required and that nutrient-specific goals will
349 be needed to reduce efforts on TSR while simultaneously reducing internal eutrophication and nutrient leaching.

350 This is especially relevant for S since we found that porewater S concentrations only increased with minimal TSR,

351 and peat type and vegetation had no influence. Our results further suggest that vegetation growth for a short period
352 of time after rewetting may be a solution to limit nutrient availability on near-neutral peat, which is in line with
353 earlier findings on near-neutral and acid peat (Vroom et al. 2022).

354 *4.3 Main drivers of CH₄ emissions*

355 Various factors are known to affect CH₄ emissions, e.g., substrate availability (labile carbon), pH, alkalinity,
356 porewater (micro)nutrients, and redox potential. These mechanisms can be categorized as CH₄-production
357 stimulating (e.g., pH and alkalinity), CH₄-production limiting (e.g., SO₄²⁻), or CH₄ consuming (e.g., anaerobic CH₄
358 oxidation) (Table 1). Porewater alkalinity was strongly associated with CH₄ emissions in both experiments and to a
359 lesser extent porewater pH. Minimal TSR decreased both porewater alkalinity and pH. Studies performed in
360 peatlands indicate the suppression of CH₄ production in acid conditions (Williams and Crawford 1984; Smolders et
361 al. 2002; Ye et al. 2011) and report an optimal pH for methanogenesis between 6.0 and 7.0 (Williams and Crawford
362 1984; Blodau 2002; Nilsson and Öquist 2009). Therefore, the drop in pH due to TSR may have affected CH₄ fluxes,
363 but it is unlikely to be the dominant factor. Acid and poorly buffered conditions may also favor peat accumulation,
364 meaning that low alkalinity values, usually observed in less reactive systems, may hamper mineralization (Roelofs
365 1991; Smolders et al. 2006). This is supported by the simulated CH₄ production when bicarbonate is added to a
366 system (Harpenslager et al. 2015). After inundation alkalinity (bicarbonate) is generated internally due to the
367 anaerobic mineralization of labile organic matter (van der Heide et al. 2010). Since porewater elements and nutrients
368 seemed to be only loosely associated with CH₄ emissions, it is suggested that substrate availability seemed to be the
369 dominant controller of methanogenic activity in our study.

370 We found that cumulative root biomass, used here as a proxy for easily decomposable or labile carbon, was also
371 highly associated with CH₄ emissions. Although root biomass data was not available for experiment 2, we assume
372 that the rooting depth of grasses remained similar over the course of two years in the same paddock sampled for
373 experiment 1. Similarly, Pypker et al. (2016) found that increased plant productivity observed in summer favored
374 CH₄ production, due to the enhanced input of labile carbon. The top layer of the peat, which is typically oxic,
375 receives most of the labile carbon (Artz 2009; Hahn-Schöfl et al. 2011; Hahn et al. 2015). Especially decaying
376 aboveground and belowground biomass have been associated with peak CH₄ emissions (Hahn-Schöfl et al. 2011;
377 Sibiyana and Muzenda 2014; Franz et al. 2015). A similar result was found by Helfter et al. (2022), where the higher

378 green vegetation and aboveground biomass during the summer resulted in higher CH₄ emissions. Noteworthy,
379 Girkin et al. (2018) showed that the composition of root exudates can be more important as regulators of CO₂ and
380 CH₄ production than their input rate. In addition, when roots decay (i.e., loss of labile carbon), a rapid turnover to
381 more recalcitrant compounds occurs (Artz 2009; Glaser and Chanton 2009; Hahn-Schöfl et al. 2011). Accordingly,
382 we argue that the reduction of CH₄ emissions observed with TSR is mainly related to the removal of labile carbon
383 from the system. The substrate argument is further supported by the initial increase followed by a decrease in GHG
384 emissions over time when less than 15 cm of topsoil was removed. Similarly, the highest CO₂ emissions arise with 5
385 cm TSR suggesting high carbon turnover. In top layers, methanogenic and heterotrophic microorganisms could use
386 the large carbon pool, whereas deeper TSR would result in a cut-off from the labile carbon resulting in suppression
387 of CH₄ emissions (Segers 1998). The CH₄ emissions observed in the treatments with deeper TSR are probably
388 related to low to very low substrate availability limiting methanogen activity deeper in the profile (Yrjälä et al. 2011;
389 Urbanová and Bárta 2016).

390 Although low amounts of CH₄ are still being emitted after almost two years, we expect that high emissions would
391 occur if a new pool of fresh carbon enters the system, e.g., as a consequence of plant or algae growth (Hahn-Schöfl
392 et al. 2011; Harpenslager et al. 2015). That could be an important reason to remove the nutrient-rich layer rather
393 than only the top 5 cm, resulting in largely lower productivity and, consequently, biomass production and labile
394 organic matter. However, we also showed that 5 cm TSR reduced nutrient concentrations in the IN peat, and
395 therefore, we argue that an assessment of labile carbon in the top peat layer prior to peat treatment (i.e., TSR and
396 rewetting) can save resources and reduce environmental impacts. This assessment can make use of porewater CH₄
397 concentrations since it showed positive correlations with CH₄ emissions on both peat types and with and without the
398 presence of *T. latifolia*. In addition, both CH₄ emissions and CH₄ porewater were similarly affected by TSR. This
399 suggests that the effects we observe on emissions are likely the result of increased CH₄ production. In turn,
400 differences in the transport mechanism and CH₄ oxidation in the water layer are less likely to explain the TSR effect.

401 **Table 1.** Summary of relevant CH₄ flux mechanisms. The table was limited to the elements investigated in the present study.

Category	Element(s) involved	Mechanism	Proof	Supporting refs
CH ₄ -production stimulating	pH	Optimal production at pH 6.0 to 7.0	Similar trend to CH ₄ flux (i.e., pH decreased with increasing TSR)	1, 2, 3
	Alkalinity	Stimulates mineralization	Similar trend to CH ₄ flux (i.e., alkalinity decreased with increasing TSR)	4, 5, 6
	Labile C	Substrate for methanogenesis	Similar trend to CH ₄ flux (i.e., labile C decreased with increasing TSR)	2, 6, 7, 8, 9
	NH ₄ ⁺	Increases mineralization of labile carbon and limits CH ₄ oxidation by binding to receptors of methanotrophs	Similar trend to CH ₄ flux (i.e., NH ₄ ⁺ reduced with increasing TSR)	10, 11, 12, 13
	Fe ³⁺	Increased mobilization results in organic matter breakdown	Increasing concentrations over the first three months as the CH ₄ fluxes	14
CH ₄ -production limiting	SO ₄ ²⁻	Competitive advantage SO ₄ ²⁻ over CO ₂ reducers and sulfide toxicity	Opposite trend to CH ₄ flux (i.e., S increased with increasing TSR)	15, 16, 17, 18, 19
CH ₄ -consuming	SO ₄ ²⁻ and Fe ³⁺	Enables anaerobic CH ₄ oxidation	Different trends compared to CH ₄ flux (i.e., S increased with increasing TSR, and Fe concentrations were not affected as CH ₄ by TSR)	20, 21, 22, 23, 24
Ambiguous	P	Stimulates microbial growth (producers and oxidizers)	P concentrations were not affected as CH ₄ by TSR.	25, 26, 27
References 1 Williams and Crawford 1984; 2 Smolders et al. 2002; 3 Ye et al. 2011; 4 Roelofs 1991; 5 Smolders et al. 2006; 6 Harpenslager et al. 2015; 7 Segers 1998; 8 Conrad 1999; 9 Conrad 2007; 10 O'neill and Wilkinson 1977; 11 King and Schnell 1994; 12 Bodelier and Laanbroek 2004; 13 Currey et al. 2010; 14 Emsens et al. 2016; 15 Maillacheruvu et al. 1993; 16 Gauci et al. 2004; 17 Gauci et al. 2005; 18 Blodau et al. 2007; 19 De Jong et al. 2020; 20 Smemo and Yavitt 2007; 21 Zhu et al. 2012; 22 Wegener et al. 2015; 23 Ettwig et al. 2016; 24 Cai et al. 2018; 25 Schrier-Uijl et al. 2011; 26 Medvedeff et al. 2014; 27 Nijman et al. 2022				

403 **5 Conclusions and implications for management**

404 The present study shows that i) there is a large variation in GHG emissions depending on TSR and time since
405 rewetting; ii) 5 to 10 cm TSR may be sufficient to greatly reduce GHG emissions upon rewetting; iii) these climate
406 benefits are not offset by introducing wetland vegetation (i.e., *T. latifolia*), which in turn further reduces emission in
407 the first period after rewetting and bypasses CH₄ emission peaks three months after rewetting; and iv) 5 cm TSR
408 may not be enough to reduce nutrient availability. These results indicate that costs can be reduced by minimizing
409 TSR prior to peatland rewetting (e.g., transport, labor, machines), when reducing GHG emissions and minimizing
410 soil subsidence is the main goal. It is of paramount importance that the area is not ploughed, as this homogenizes the
411 vertical profile. In this light, current standard TSR practices of 30-60 cm are not required from a climate perspective
412 but will, in many cases, be necessary from a nutrient perspective (Van Diggelen et al. 2020). Therefore, clear targets
413 need to be set before determining TSR depth. Furthermore, as GHG emission after rewetting is largely driven by
414 labile carbon, we suggest rapid rewetting after TSR to prevent the establishment of vegetation that enriches the
415 topsoil with labile carbon (e.g., plant litter, roots, exudates, aboveground biomass). Further research is needed to
416 develop a tool to easily predict optimal TSR depth to reduce GHG emissions for all types of peatlands (e.g.,
417 differing in nutrient/mineral status) in temperate and boreal climates.

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618 **Statements and Declarations**

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627 **Competing Interests**

628 The authors have no relevant financial or non-financial interests to disclose

629 **Data Availability**

630 Data will be stored in DANS-EASY upon acceptance.