1 **Spatial micro-distribution of methanotrophic**
2 **activity along a 120-year afforestation**
3 **chronosequence**

4 Saeed Karbin¹, Frank Hagedorn², David Hiltbrunner², Stefan Zimmermann² and
5 Pascal A. Niklaus¹

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- 10
11
12 1) Institute of Evolutionary Biology and Environmental Studies, Winterthurerstrasse
13 190, CH-8057 Zürich, Switzerland
- 14 2) Swiss Federal Institute for Forest, Snow and Landscape Research WSL
15 Zürcherstrasse 111, CH-8903, Birmensdorf, Switzerland

16
17 Correspondence: Pascal.Niklaus@ieu.uzh.ch

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Abstract

Aims. Methanotrophic bacteria drive upland soil methane (CH₄) uptake. Land-use change often affects their activity, but the mechanisms involved are not well understood. We studied soil-atmosphere CH₄ fluxes along a 120-year Norway spruce afforestation chronosequence on subalpine pasture, testing whether effects were related to shifts in the spatial niche of methanotrophs. Previous field data had shown that soil ¹⁴CH₄ uptake increased with forest age, and that this effect was driven by decreased water filled pore space due to higher rainfall interception in the more developed canopies of older forest stands.

Methods. The spatial distribution of methanotrophic activity was determined by ¹⁴CH₄-labelling followed by soil section preparation, aggregate size fractionation, aggregate erosion, and micro-autoradiographic imaging.

Results. Uptake rates of CH₄ measured in laboratory incubations of soil cores as well as their water contents largely followed the in situ measurements previously made in the field. ¹⁴CH₄ assimilation was heterogeneously distributed, and occurred further down the soil profile in older forest that had a more developed organic layer that did not contribute to CH₄ uptake. Assimilation was largest in 2–8 mm aggregates, and higher at the exterior than in the interior of aggregates.

Conclusions. Our data indicates that differences in soil aggregation and related methanotrophic activities did not contribute substantially to higher CH₄ uptake in older forest, mostly because aggregation did not change much with age. On a per mass basis, however, large aggregates contributed less to CH₄ uptake due to their unfavorable surface to volume ratio. More generally, we argue that the (sub-)aggregate heterogeneity of soil microbial activity and diversity is underexplored, although it critically determines ecological interactions that drive ecosystem-level processes.

Keywords: methanotrophic bacteria, soil aggregation, afforestation, ¹⁴C labeling, land-use changes, spatial distribution of microbial activity

Introduction

Anthropogenic activities including ruminant livestock farming, fossil fuel exploration, and rice cultivation have increased atmospheric methane (CH_4) concentrations above pre-industrial levels. CH_4 currently is the most important anthropogenic greenhouse gas after CO_2 , with key components of the global CH_4 cycle being mediated by soil micro-organisms (IPCC, 2013). At low redox potential, e.g. in water-logged soils, methanogenic archaea produce CH_4 . In contrast, oxic upland soils often are a net sink for atmospheric CH_4 due to the activity of methanotrophic bacteria. Methanotrophs can also oxidize CH_4 produced by soil-internal methanogenesis, so that they functionally act as a filter that reduces the amount of CH_4 that would otherwise be released to the atmosphere. Methanotrophic activity is environmentally beneficial because methanotrophs convert CH_4 -carbon to organic forms or to CO_2 which has a much lower greenhouse warming potential than CH_4 .

Upland soil often show no or only little methanogenesis, and CH_4 oxidation follows a “high affinity” kinetic. The nature of the methanotrophs driving the uptake of atmospheric CH_4 in these systems remains unclear, and methanotrophs able to grow under atmospheric CH_4 concentrations have not been isolated to date. DNA sequence data suggest that these are distinct from the communities found in systems with important soil-internal sources and thus periodically higher soil CH_4 concentrations (Dunfield, 2007), but recent studies have shown that flush feeders can temporally exhibit high-affinity kinetics (Baani and Liesack, 2008; Cai et al., 2016).

Irrespective of the identity of methanotrophs, CH_4 uptake by upland soils significantly changes with land-use. Forests generally oxidize larger amounts of atmospheric CH_4 than grassland (Boeckx et al., 1997; Gundersen et al., 2012; Tate et al., 2007), and CH_4 uptake by arable land often is even lower than in grassland (Barcena et al., 2014; Smith et al., 2000). As a general rule, conversion of forest to agricultural land reduces the soil CH_4 sink, whereas reverting arable or pasture systems back to forest increases soil CH_4 uptake (Menyailo et al., 2010; Menyailo and Hungate, 2003). Chronosequence and paired site studies suggest that the recovery of the soil CH_4 sink can take decades to centuries (Prieme et al., 1997; Smith et al., 2000), for reasons not well understood. Low recovery rates may be related to the presumably very slow growth rates of methanotrophic communities thriving mostly on

atmospheric CH₄, but indirect effects via nutrient dynamics or re-adjustments of soil structure may also be at play (Conant et al., 2004; Duchicela et al., 2013; Spohn and Giani, 2011).

Several mechanisms have been proposed to explain the dependence of the soil CH₄ sink on land use. First, the decline in CH₄ uptake in agricultural land may be related to the application of mineral nitrogen fertilizers. In particular ammonium-based fertilizers have been shown to decrease soil CH₄ uptake (Hütsch et al., 1993), possibly by direct inhibition of CH₄ assimilation at the enzymatic level (Carlsen et al., 1991; Dalton, 1977). While this mechanism has been demonstrated under laboratory settings (Bedard and Knowles, 1989), it is less clear whether it also is responsible for such effects under field conditions (e.g. Barcena et al., 2014; Bodelier and Laanbroek, 2004). Second, soil CH₄ sinks may also decrease in response to altered soil structure during land-use change (Abichou et al., 2011; Ball et al., 1999; Hütsch, 1998). Soil properties such as water content, pore size distribution, and gas diffusivity can alter methanotrophic activity and are related to soil aggregate structure (Kasper et al., 2009). Methanotrophs have been shown to inhabit particular soil domains (Boeckx and van Cleemput, 2001; Stiehl-Braun et al., 2011a), and soil CH₄ uptake can decline when aggregation is lost (Abichou et al., 2011), suggesting that the ecological niches of methanotrophic bacteria are linked to the occurrence of macro-aggregates. However, similar effects may also be due to a reduced number of macro-pores and resulting higher water filled pore space, which likely limits diffusive methane transport (Castro et al., 1994). In the long term, soil structural changes can also lead to shifts in methanotrophic community composition (Kumaresan et al., 2011; McNamara et al., 2008; Singh et al., 2007).

CH₄ uptake by soils may also vary at the sub-aggregate scale, e.g. radially within aggregates. The exterior and interior of aggregates differ in physico-chemical characteristics and substrate availability, thus providing distinct microhabitats for microbial communities (e.g. Wilcke et al., 1999). Using microsensors, Hojberg et al. (1994) observed strong gradients in O₂ and N₂O concentrations from the surface to the core of soil aggregates. Based on ¹⁴C imaging of assimilated ¹⁴CH₄ in soil cores embedded in resin, Stiehl-Braun et al. (2011a) suggested that CH₄ uptake takes primarily place on aggregate surfaces, but their results were inconclusive because aggregate surfaces could not unequivocally be identified in their soil sections. However, this spatial niche would allow

methanotrophs the best access to atmospheric CH₄, but would expose them to more severe environmental fluctuations (e.g. drought) and microbial grazing.

Given the extreme small-scale heterogeneity of upland soils, identifying the small-scale spatial niche of methanotrophs is a key prerequisite to understand their ecology and to develop a mechanistic understanding of microbial CH₄ transformations. To date, related investigations have been hindered because tools allowing to study methanotrophic activity in a small-scale context are largely lacking, in part because they cannot be cultivated and their nature thus remains in part elusive. We have previously studied soil-atmosphere CH₄ flux rates in an afforestation chronosequence in which Norway spruce (*Picea abies* L.) was established on a former subalpine pasture (Hiltbrunner et al., 2012). Field-measured soil CH₄ uptake increased with stand age, and we speculated that these fluxes were primarily driven by the volumetrically drier soils and decreased water filled pore space that we had found. Here, we present further investigations in which we analyzed soil structural changes and determined the spatial micro-distribution of soil CH₄ uptake. This was achieved by ¹⁴CH₄ labelling of soil cores, followed by autoradiographic analysis of intact soil sections. We further physically fractionated these soil cores and eroded aggregates to determine the spatial niche of the active methanotrophs in relation to aggregate structure. We hypothesized aggregation would increase in older forest stands, an effects that might be driven by larger soil carbon input rates and an increased abundance of ectomycorrhizal fungi. At the micro-scale, we expected a distinct gradient of methanotrophic activity within aggregates with the highest activity in the exterior of soil aggregates due to larger CH₄ availability.

Materials and Methods

Site description

We studied the spatial distribution of methanotrophic activity along a sub-alpine afforestation chronosequence in Switzerland (Jaun pass area, Canton of Fribourg, 46°37'17 N; 7°15'54 E). The site is located on a south-facing slope extending from 1450 to 1700 m a.s.l. that has been used as pasture for probably several centuries but certainly for the past 150 years. After severe avalanches in 1956, an area of about 15 ha on the eastern part of the slope was gradually afforested with Norway spruce (*Picea abies* L.), while the western part remained as a pasture. Separate patches of forest were planted in different years, resulting in stands 25, 30, 40, 45, and >120 years old (see Hiltbrunner et al., 2012, for more details).

Soils are Eutric Cambisols on calcareous bedrock. On average, soils were 80 cm deep and carbonate-free to a depth of 60 cm. The mineral A horizon contained 18.7±2.1% sand, 31.2±0.7% silt (2—63 µm), and 50.1±1.8% clay (mean ± standard error of a total of 20 measurements). We refer to Hiltbrunner et al. (2013) for a more detailed description of the soil profile including the distribution of organic carbon. See Table 1 for climatic conditions.

Soil sampling and ¹⁴CH₄ labelling

In September 2011 and again in September 2012, intact soil cores (20 cm depth x 6.5 cm internal diameter) were sampled in PVC tubes. Loose organic material, i.e. any O_L horizon, was removed. We then pre-cut the top soil along the tube's circumference with a knife to minimize soil compaction and then drove the PVC tubes 15 cm into the soil. The A horizon was approximately 20 cm deep so that the cores covered O_F, O_H, and the top of the A horizon. The tubes were excavated from the side, capped at both ends to prevent water loss, and transported upright to the laboratory.

We incubated the soil cores (still capped at the bottom end) in air-tight 3 L jars. Soil net CH₄ uptake was determined by collecting three headspace samples at hourly intervals (Agilent 7890N gas chromatograph equipped with a flame ionisation detector, Wilmington, Delaware, USA). Soil-

atmosphere CH₄ flux rates were determined by linear regression of headspace concentrations vs. sampling time.

Thereafter, the jars were first ventilated for about 15 min and then radio-labelled by manually injecting ¹⁴CH₄ into the jars, in portions, using a syringe. We maintained headspace concentrations in the range of 5-8 μL CH₄ L⁻¹ (analysis by gas chromatography) for a total of 7 days. This was achieved by adding ¹⁴CH₄ when required. Once a total of ~100 kBq had been injected, we continued with the same procedure but adding unlabelled CH₄. The incubation jars also contained plastic tubes with 100 mL 1.5 M NaOH to prevent secondary fixation of ¹⁴CO₂ released by microbial respiration during the incubation. O₂ was regularly injected into the jars to maintain aerobic conditions (15–20% O₂).

Autoradiographic imaging of intact soil cores

Two labeled soil cores per sampling date and plot were freeze-dried and impregnated with epoxy resin (Laromin C 260, BASF, Ludwigshafen, Germany, mixed at a ratio of 2:3 with Araldite DY 026SP hardener, Astorit AG, Einsiedeln, Switzerland). Resin penetration was improved by evacuating the soil cores with resin to an end pressure of 25 kPa, and slowly bringing the core back to atmospheric pressure. After about 2–3 d of curing at room temperature, the resin was fully hardened overnight at 60 °C. Then, the soil cores were cut twice length-wise and divided in three sections that were each mounted on 5×5 cm glass slides. The section's surfaces were leveled with a diamond cup mill (Discoplan, Struers GmbH, Birmensdorf, Switzerland) and used to expose phosphor imaging plates (BAS III S, Fuji Photo Film Ltd., Tokyo, Japan) for 3 d. The imaging plates were scanned by red-excited blue fluorescence at a resolution of 200 μm and the three slides representing a soil core recomposed using a Matlab-script that made use of the Image Processing Toolbox (Mathworks, Natick, MA). Recomposition was achieved by determining a transformation matrix (combination of translation and rotation) for each image part, based on the visual identification of control points that matched features opposite the line along which the slides had been separated. The label distribution was then explored visually and the vertical distribution of ¹⁴C determined by averaging pixel intensities per horizontal pixel line. Areas with large stones were excluded for this procedure.

Autoradiographic imaging of isolated soil aggregates

We isolated soil aggregates 2 to 12 mm in size from a separate labelled soil core per plot, attempting to minimize mechanical disturbance (September 28, 2011 sampling). These aggregates were placed in petri dishes and impregnated with epoxy resin as described previously for intact soil cores. Autoradiographies were obtained similarly.

Aggregate fractionation

We eroded labeled soil aggregates mechanically to separate exterior and interior fractions slightly modifying the method described by Wilcke et al. (1999). First, labeled soil cores were divided into 0-6, 6-12 and 12-16 cm depth segments. Then, the soil depth segments were separated into aggregate size fractions (>16, 16-8, 8-4, 2-4, <2mm) by sieving through a series of sieves, applying only minimal mechanical energy. Each size fraction was weighed and its water content was determined gravimetrically (5 g subsample, 105°C). Approximately 300 g of soil aggregate size fractions were then frozen by immersion in liquid nitrogen. Then, material was eroded by repeated immersion in distilled water, which melted a thin layer on the outside of the aggregates, followed by shaking on a 2 mm sieve, which removed this layer. To erode approximately equal amounts of soil in each aggregate size fraction, the sieve was shaken 75, 60, 50 and 50 times for the >16, 16-8, 8-4 and 2-4 mm fraction, respectively. In the course of this procedure, aggregates dispersed from the exterior to the interior, with the eroded fractions collected in a water bowl underneath the sieve. The solid eroded material was obtained by transferring the suspension into a long glass tube adding a flocculation agent (2 mL 1 M MgSO₄) and pipetting the water above the settled soil material after 24 hours. All soil fractions were dried at 105°C. A subsample was ground in a mortar, oxidized (200 mg material, A307 sample oxidizer, Perkin Elmer, Waltham, MA) and its ¹⁴C content determined by liquid scintillation counting (TRI-2900TR, Perkin Elmer, Waltham, MA). Soluble ¹⁴C remaining in the supernatant of the solution used for aggregate erosion was determined similarly (Ultima Gold scintillation cocktail, Perkin Elmer).

Statistical analysis

Data were analyzed by fitting linear models reflecting the design of the study. We fitted effects of land use type (forest vs. grassland), forest stand age (log-transformed), and altitude. Since altitude and stand age were not orthogonal (Hiltbrunner et al., 2012), we fitted models correcting for altitude-effects before and after stand age to estimate the degree of confounding. When several measures per plot were analyzed (e.g. repeated measures or analyses including several soil layers), linear mixed effects models were fitted (ASReml, VSN International, Hempel-Hempstead, UK) which included the additional random effects plot, and, where appropriate, plot $\times$ year. These terms are necessary to ensure proper replication of significance tests. For the analysis of activity by soil layer, data were averaged by 1 cm soil layers and a first order autoregressive spatial correlation structure between soil layers included in the analysis to account for non-independence of residuals from the same soil core and plot.

Results

CH₄ uptake by soil cores

Net CH₄ uptake of soil cores did not differ significantly between grassland and forest, but increased with forest stand age (Fig. 1, linear model correcting for altitude; $P=0.06$ for joint analysis of both years; $P=0.03$ when 2012 data analyzed separately; n.s. for 2011). Volumetric soil moisture was significantly higher in pasture than in forest soil (Fig. 2, $P<0.01$, joint analysis for both years), but no effect of forest age was detected.

¹⁴CH₄ assimilation in soil aggregate fractions

The soils investigated were well-aggregated, with 10% of the soil material recovered in the <2mm fraction, 20% in both the 2-4 and 4-8 mm fraction, 38% in the 8-16 mm fraction, and 11% in the >16mm fraction (Fig. 3). The size of the largest fraction was sensitive to soil handling during the sieving process and more variable; the statistical analyses were therefore restricted to the small fractions. Average aggregate size increased with depth, both in grassland and forest ($P<0.001$ for size $\times$ depth), and decreased with forest stand age ($P=0.02$ for size $\times$ age), and more so in deeper soil layers ($P<0.01$ for size $\times$ age $\times$ depth).

The concentrations of ¹⁴C, reflecting net ¹⁴CH₄ assimilation, were generally higher in small than in large aggregates (Table 1; $P<0.001$), irrespective of land use, forest age, and soil depth. Nevertheless, a larger total fraction of the applied label was found in larger aggregate classes because these made up a larger mass fraction (Table 2). The eroded exterior of the aggregates contained approximately twice as much ¹⁴C per unit mass than the stable interior in all aggregate classes (Fig. 4).

Spatial distribution of ¹⁴C in soil sections

Net assimilation of ¹⁴C in soil was heterogeneous (Figs. 5 and 6), with increased activities on the surface of aggregates. While aggregate surfaces are difficult to identify in intact sections, this finding was confirmed by the autoradiographies of isolated aggregates (Fig. 7). The ¹⁴C-depth profiles differed between grassland and forest (Fig. 8, $P<0.001$), with mean ¹⁴CH₄ assimilation depth (i.e. depth

230 weighted by ^{14}C content) about 1 cm closer to the soil surface in grassland ($P < 0.01$). Within the forest
231 plots, the amount of ^{14}C assimilated in the layers near the soil surface decreased with stand age (Figs.
232 5 and 6; $P < 0.05$ for layer $\times$ age), and mean ^{14}C depth therefore increased with age ($P < 0.05$).

Discussion

Along the afforestation chronosequence investigated here, we have measured soil-atmosphere fluxes of CH₄ in a previous study (Hiltbrunner et al., 2012) and found that net soil CH₄ uptake increased with forest stand age and that this effect correlated negatively with soil moisture and water filled pore space. Bulk density and soil porosity did not explain this pattern, and we concluded that the observed increase in soil CH₄ uptake was most likely driven by higher rainfall interception in the older forest canopies (Hiltbrunner et al., 2012). However, soil CH₄ fluxes are also affected by a multitude of other drivers which are frequently linked to soil structure (Hütsch, 1998; Shukla et al., 2013). Our ¹⁴C based analysis of the micro-scale distribution of methanotrophic activity revealed a heterogeneous distribution in soils, with higher activities at the exterior than in the interior of soil aggregates. The active CH₄-assimilating zone shifted downwards the soil profile with increasing stand age; this effect was due to the development of an organic layer (O_F and O_H horizon) that did not contribute to CH₄ uptake.

Soil-atmosphere fluxes of trace gases are difficult to measure under laboratory conditions, and often deviate from in-situ assessments (Abichou et al., 2011; Hiltbrunner et al., 2012). Reasons include altered environmental conditions (e.g. temperature profiles and gas transport), effects of disturbance (e.g. compaction, removal of live plant roots), and in the case of soil cores also edge effects (e.g. facilitated diffusion along the core walls). Our laboratory incubations were therefore not intended as substitute for field measurements but to confirm that conditions largely reflected the patterns we had found in the field one year before (Hiltbrunner et al., 2012). The observed increases in CH₄ oxidation in forest relative to grassland are in agreement with the findings in temperate pine (Peichl et al., 2010) and hardwood forests (Menyailo and Hungate, 2003), but in contrast to Christiansen and Gundersen (2011) who reported decreasing CH₄ uptake in spruce forest. In a Danish 200-year chronosequence of oak and larch, Barcena et al. (2014) found increasing abundances of methane oxidizing soil bacteria with stand age. In our study, methanotrophic activity shifted downwards with forest soil development, an effect that is most likely related to the buildup of an organic layer which showed no substantial CH₄ oxidation. This finding is compatible with other

studies in which CH₄ oxidation was low or absent in the organic layer and concentrates in the top layers of the mineral soils (e.g. Adamsen and King, 1993; Bradford et al., 2001). Also, methanotrophic activity can be inhibited by monoterpenes (Amaral et al., 1998) and organic acids (Wieczorek et al., 2011), processes which may be more important in the organic layer of mature forest stands. Oxidation rates of atmospheric CH₄ generally are limited by diffusion rates due to the low CH₄ concentrations; a downward shift of activity thus would tend to decrease soil CH₄ uptake rates. In the present study, however, such an effect was not evident, probably because the diffusive resistance of the relatively well-aerated organic layer was low. This situation is comparable to the findings of Stiehl-Braun et al. (2011a) who reported that fertilizer application inhibited methanotrophic activity in top soil layers of a grassland but that the inhibition had no effect on soil-atmosphere CH₄ exchange – at least when soils were dry. In the afforestation chronosequence we studied, soils in older forest stands also were drier because the taller forest canopies intercepted more rainfall and the organic layer had a greater pore space. Therefore, if there would have been a limiting effect of the downward shift in methanotrophic activity, it might have been compensated by a lower soil moisture and a greater CH₄ diffusion into the mineral soil.

Several studies reported a relation between soil CH₄ uptake and nitrification. For example, Reay et al. (2005) found low CH₄ uptake in the top organic layer of Alder stands. Low CH₄ oxidation was associated with high nitrification rates. CH₄ oxidation rates were higher and nitrification rates lower in stands of other species, including Norway spruce, the species planted in our study. Potential nitrification rates generally are largest near the surface of forest soils, and generally decrease with depth (Laverman et al., 2000). How this relates to the inhibition of high affinity CH₄ oxidation is more complicated, because nitrification on one hand removes NH₄⁺ (or NH₃), a compound known to inhibit CH₄ oxidation by competing for the active site of methane mono-oxygenase, but on the other hand nitrification often is an indication of high NH₄⁺ concentrations, even if these are not found in bulk soil measurements because these are restricted to episodic events or small domains within soil structure. Also, intermediate products of nitrification (e.g. NO₂⁻) may have detrimental effects on methanotrophs (King and Schnell, 1994; Schnell and King, 1994). Finally, mineral N concentrations sometimes also are positively correlated to the soil CH₄ sink (Bodelier and Laanbroek, 2004). We do not have a

detailed analysis of nitrogen cycling for our site; however, at our site, Hiltbrunner et al. (2012) did not find changes in nitrification rates with forest stand age, and it therefore appears unlikely that nitrification was an important driver of the age effects we observed.

Small-scale heterogeneity in soil methanotrophic activity has been reported in previous studies that adopted autoradiographic techniques (Karbin et al., 2015a; Karbin et al., 2015b; Rime and Niklaus, 2017; Stiehl-Braun et al., 2011a; Stiehl-Braun et al., 2011b). While we have argued that this heterogeneity was related to aggregate structure, soil structure was not unambiguously identifiable once the soil cores were embedded in resin. The enhanced activities in eroded aggregate surfaces now demonstrate that indeed methanotrophic activity is concentrated in the aggregate's exterior and is largely absent in their center. Very likely, this pattern can be attributed to diffusion limitations (Hojberg et al., 1994). On the other hand, locations towards the aggregate core would protect methanotrophs from adverse biotic and abiotic effects such as protozoan grazing, drought stress, freeze-thaw events, and possibly also chemical inhibition by organic compounds such as terpenes (Amaral et al., 1998). Such adverse effects could be important given the oligotrophic nature of methanotrophs in low CH₄ environments which implies a low resilience after disturbance.

We found the largest concentrations of label from ¹⁴CH₄ assimilation in the smallest aggregates, irrespective of forest age. As soil aggregate volume increases, air diffusion into the central part of soil aggregate decreases (Sextstone et al., 1985), and CH₄ availability will become restricted. Small aggregates have a larger surface to volume ratio, which may explain why the ¹⁴C label decreased with size. In our study, comparing aggregation among forest ages was difficult because we sampled by depth rather than by horizon. However, our data suggest that aggregation did not change much with age. In other studies, increasing fractions of macro-aggregates were found under afforestation (Wei et al., 2013; Wu et al., 2013), but these studies focused on much smaller aggregates (macro aggregates were defined as exceeding a diameter of 0.25 mm) and soils had a long history of tillage. Interestingly, aggregates smaller than 2 mm showed lower ¹⁴CH₄ uptake in our study.

In conclusion, our results indicate an increased soil CH₄ uptake in older forest stands, which can be related to lower soil moisture due to interception and possibly also to a larger share of aggregates showing a higher than average methanotrophic activity. Our analysis of aggregate sizes and fractions

316 indicates small-scale heterogeneity in the distribution of methanotrophic activity that follows a radial
317 gradient with the highest activities at the exterior of aggregates. It would be promising to analyze,
318 using molecular methods, whether this gradient in activity coincides with a gradient in methanotrophic
319 abundance. More generally, the small-scale (sub-aggregate) spatial heterogeneity of soil microbial
320 activity and diversity currently appears underexplored, although it may have important implications
321 for our understanding of the ecological mechanisms that control microbially-mediated processes at the
322 ecosystem-level.

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Tables

Table 1. Climatic conditions at the field site. Data are monthly means and their standard deviations for the 1960-2012 period, for an elevation of 1650 m a.s.l. Data were interpolated based on data from a network of climate stations that was combined with simulations using a regional climate model (see Remund et al., 2016) for further details).

Month	Temperature (°C)	Precipitation (mm)
Jan	-2.6 ± 2.4	101 ± 71
Feb	-2.6 ± 2.7	99 ± 71
Mar	-0.6 ± 2.2	103 ± 62
Apr	2.3 ± 1.9	98 ± 46
May	6.8 ± 1.8	119 ± 42
Jun	10.0 ± 1.6	143 ± 51
Jul	12.2 ± 1.5	147 ± 51
Aug	12.0 ± 1.6	151 ± 51
Sep	9.3 ± 1.8	103 ± 48
Oct	6.0 ± 2.0	96 ± 58
Nov	0.9 ± 2.1	111 ± 73
Dec	-1.8 ± 2.2	124 ± 78

Table 2. Amount of label recovered in aggregate size fractions, expressed as total activity and concentration per dry aggregate mass. Note that the largest fraction is not well defined (see methods for details).

Size class	¹⁴ C label	
	total (Bq)	concentration (Bq g ⁻¹)
>16 mm	31639 ± 6555	481 ± 46
8-16 mm	62816 ± 11282	589 ± 36
4-8 mm	36723 ± 5497	655 ± 41
2-4 mm	44857 ± 6549	760 ± 49
0-2 mm	21097 ± 1951	931 ± 61

Figures

Figure 1: Net CH₄ uptake of soil cores collected on September 28, 2011 (left) and September 28, 2012 (right), in dependence of land use (pasture vs forest) and stand age (forest only). Error bars indicate standard errors.

Figure 2: Volumetric soil moisture in soil cores collected on September 28, 2011 (left) and September 28, 2012 (right), in dependence of land use (pasture vs forest) and stand age (forest only).

348

349 Figure 3: Soil aggregate size distribution in pasture and forest plots. Data are shown for each
 350 fractionated soil core separately. Bars indicate the average fraction across the three depth layers
 351 analyzed (0-6, 6-12, 12-18 cm).

352

Figure 4: Ratio of ^{14}C concentration in the eroded exterior part of aggregates relative to the concentration in the remaining interior part, in dependence of land use, forest stand age, and aggregate size class. Data are means $\pm$ standard errors, using plots as replicate.

358

359 Figure 5: Auto-radiographic image of soil cores harvested on September 28 2011. Darker pixels
 360 indicate stronger ^{14}C labelling. Note that these images show the relative label distribution within
 361 individual soil cores. Differences in CH_4 uptake rate among cores are not visible because the cores
 362 were labelled separately, each with an approximately equal amount of ^{14}C .

363

Figure 6: Auto-radiographic image of soil cores harvested on September 28 2012. Darker pixels indicate stronger ^{14}C labelling. See legend of Fig. 5 for details.

1cm

368

369 Figure 7: Auto-radiographic images of soil aggregates. Darker pixels indicate higher amounts of
370 assimilated ^{14}C .

371

Figure 8: Depth distribution of net $^{14}\text{CH}_4$ assimilation in soil cores harvested on September 28, 2011 (left) and September 28, 2012 (right). Activities were standardized core-wise to unity sum of all 1 cm-layers. Data shown are means $\pm$ 1 s.e., using plots as replicates.

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