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Increasing soil methane sink along a 120-year afforestation chronosequence is driven by soil moisture

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Abstract

Upland soils are important sinks for atmospheric methane (CH_4), a process essentially driven by methanotrophic bacteria. Soil CH_4 uptake often depends on land use, with afforestation generally increasing the soil CH_4 sink. However, the mechanisms driving these changes are not well understood to date. We measured soil CH_4 and N_2O fluxes along an afforestation chronosequence with Norway spruce *(Picea abies L.)* established on an extensively grazed subalpine pasture. Our experimental design included forest stands with ages ranging from 25 to >120 years and included a factorial cattle urine addition treatment to test for the sensitivity of soil CH_4 uptake to N application. Mean CH_4 uptake significantly increased with stand age on all sampling dates. In contrast, CH_4 oxidation by sieved soils incubated in the laboratory did not show a similar age dependency. Soil CH_4 uptake was unrelated to soil N status (but cattle urine additions stimulated N_2O emission). Our data indicated that soil CH_4 uptake in older forest stands was driven by reduced soil water content, which resulted in a facilitated diffusion of atmospheric CH_4 into soils. The lower soil moisture likely resulted from increased interception and/or evapotranspiration in the older forest stands. This mechanism contrasts alternative explanations focusing on nitrogen dynamics or the composition of methanotrophic communities, although these factors also might be at play. Our findings further imply that the current dramatic increase in forested area increases CH_4 uptake in alpine regions.

Keywords: afforestation, alpine regions, chronosequence, fertilization, methane oxidation, nitrous oxide, Norway spruce, soil moisture regime

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Introduction

Methane (CH_4) is produced in water-logged soils by methanogenic archaea (Boone *et al.*, 1993). In contrast, well-aerated upland soils are the most important biological sink for atmospheric CH_4 (IPCC, 2007). Soil CH_4 uptake is essentially driven by the oxidation of CH_4 by soil methanotrophic bacteria. In many soils, both processes – methanogenesis and CH_4 oxidation – take place concurrently, with the soil acting as a net source or sink depending on which process dominates.

The largest terrestrial sinks for atmospheric CH_4 are generally found in forest soils. When forests are converted into grassland or arable fields, soil CH_4 uptake generally decreases (Hütsch *et al.*, 1994; Willison *et al.*, 1995; Smith *et al.*, 2000). Many investigations have attributed this decrease in methanotrophic activity to the disturbance of soil physical structure associated with such land-use changes, and to the application of mineral nitrogen fertilizers. Physical disturbances of the soils through ploughing disrupts aggregates, which might affect the ecological niche of methanotrophs (Boeckx & Cleemput, 2001), especially in coarse-textured soils (Hütsch, 1998). The use of heavy machinery on cultivated land also compacts soils, thereby restricting diffusive transport of atmospheric CH_4 into soils (Ball *et al.*, 1997b; Smith *et al.*, 2003). Fertilization of agricultural fields, in particular with ammonium-based fertilizers, has been shown to inhibit CH_4 oxidation (King & Schnell, 1994; Gulledge *et al.*, 1997; Whalen, 2000; Jassal *et al.*, 2011); however, positive effects of N fertilization also have been reported (Bodelier & Laanbroek, 2004).

Interestingly, when cultivated land is abandoned, CH_4 oxidation reverts only very slowly to precultivation levels. Paired-site studies have demonstrated that this process can take many years (Priemé *et al.*, 1997; Smith *et al.*, 2000), but it is not well understood to date why the increase in soil CH_4 uptake is so slow. One factor involved might be the very low-growth rates of methanotrophic bacteria thriving on atmospheric CH_4 (Priemé *et al.*, 1996; King, 1997; Menyailo *et al.*, 2008). Another reason may be that the original soil structure is

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restored only after many years (Priemé *et al*., 1997; Hütsch, 1998; Smith *et al*., 2000; Regina *et al*., 2007). However, not many studies on the recovery of the soil CH_4 sink are available, presumably because not many such chronosequences have been established.

Across Europe and North America, large areas of land have been abandoned for socioeconomic reasons. In the European mountains, woody plant encroachment in abandoned grasslands is widespread (FAO, 2001). In Switzerland, the forest cover in the Alps increased by 900 km^2 between 1984 and 2005, which corresponds to a 15% increase in total forested area in this region (Brändli, 2010). Whether and to what extent soil CH_4 uptake increases under these conditions is unclear. Subalpine pastures have, generally, only moderately been grazed with little nutrient inputs and they have never been tilled. Thus, the loss of methanotrophic activity when these pastures have been established has probably been smaller than in intensified low-land pastures and arable fields (Priemé *et al*., 1997; Peichl *et al*., 2010; Christiansen & Gundersen, 2011). As a consequence, the increase in soil CH_4 uptake after afforestation might also be smaller.

In our study, we have measured soil CH_4 uptake and potential CH_4 oxidation along a chronosequence of Norway spruce afforestations spanning more than 120 years. All forest plots are located in an extensively grazed subalpine pasture. To test the sensitivity of soil CH_4 uptake to nitrogen additions, we further established a N-fertilizer treatment (cattle urine) in all chronosequence plots. Our aims were (i) to test for effects of afforestation on the soil CH_4 sink, focusing in particular on the temporal dynamics of these changes; and (ii) to test how these changes were related to changes in soil physical properties and nitrogen status.

Materials and methods

Study site and experimental design

The present study was conducted in a subalpine region in the Canton of Fribourg, Switzerland (7°15′54 E; 46°37′17 N), on a south-facing slope extending from 1450 m a.s.l to 1700 m a.s.l. This slope has been used as pasture for the last 150 years; no land-use records are available prior to this period, but it seems likely that the slope has been under pasture for several centuries. Mean summer and winter air temperatures are 11.4 °C and 0.6 °C, respectively; mean annual precipitation averages 1250 mm with a maximum in summer. Soils are Cambisols on calcareous bedrock.

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 (Fig. 1). Separate patches of forest were planted on different dates, resulting in stands 25, 30, 40, 45 and >120 years old. We established one 15 × 15 m plot in each forest patch, plus an additional four similarly sized plots in the adjacent pasture. Within each plot, four pairs of subplots were established. One randomly selected subplot per pair was treated with synthetic cattle urine, while the other one served as unfertilized reference. The synthetic cattle urine was prepared according to Fraser *et al.* (1994) and contained urea as the main N source plus glycine representing the amino acid fraction in the cattle urine, potassium bicarbonate, potassium bromide, potassium chloride and potassium sulphate. The synthetic urine solution was applied to the subplots at a rate of 20 g N m^{-2} (as 3.35 L m^{-2} aqueous solution) on August 12, 2010. The same amount of water was added to the unfertilized control plots.

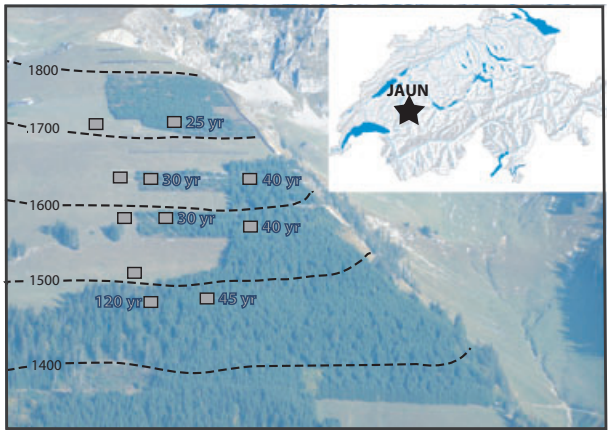

Fig. 1 Photograph of the study site showing the plots where the CH_4 and N_2O fluxes have been measured (grey squares) and the age of the respective forest stands.

Soil-atmosphere CH_4 and N_2O fluxes

Soil-atmosphere fluxes of CH_4 and N_2O were measured using static chambers. On May 17, 2010, a 32 cm diameter × 30 cm tall static chamber was lowered 20 cm into the soil of each subplot and remained there until the end of the growing season. The chambers were placed at some distance from the tree stems to avoid coarse roots. The remaining headspace volume of each chamber was determined by measuring its height aboveground at several locations within the chamber. Soil-atmosphere trace gas fluxes were determined on July 17, August 10, 13 and 20, September 3 and October 1, 2010, by closing the chamber with a gas-tight lid and sampling the headspace through a septum after 5, 20 and 35 min. The headspace samples were injected into pre-evacuated exetainers and analysed for CH_4 and N_2O concentrations using a gas chromatograph (Agilent 7890 fitted with a flame ionization (CH_4) and an electron-capture detector (N_2O), Agilent Technologies Inc., Santa Clara, CA, USA). CH_4 and N_2O flux rates were calculated by linear regression of measured concentrations against sampling time. Estimates with regression coefficients $r^2 < 0.8$ were excluded except when fluxes were close to zero.

Soil surface temperature (0–2 cm) and volumetric water content (0–15 cm) were measured concomitantly with the gas

measurement using a thermometer and time domain reflectometry (TDR) probes (TRIME-FM, IMKO, Ettlingen, Germany).

We further measured potential CH_4 oxidation rates of sieved soil under standardized laboratory conditions. On September 28, 2011, two soil cores were taken from each subplot and divided into the 0–5, 5–10, 10–15 and 15–20 cm depth layer of the mineral soil horizon. The soil fractions were sieved (2 mm mesh size), and fresh soil equivalent to 100 g dry weight placed into gas-tight jars fitted with a septum. The soils were equilibrated at 20 °C overnight; then, the jars were aerated for 30 min, closed again; and CH_4 oxidation rates were determined by measuring headspace CH_4 concentrations after 5, 125 and 425 min. These incubations were conducted under atmospheric CH_4 concentrations, i.e. no extra CH_4 was injected into the headspace.

Soil bulk density and porosity

On November 8, 2011, three soil cylinders of 10.8 cm diameter × 11 cm depth were collected per plot. Bulk soil density was estimated by dividing the mass of the dried soil (105 °C) by the volume of the cylinder. Particle density was determined by the pycnometer method (Blake & Hartge, 1986). Total porosity was calculated as 1-(bulk density/particle density). Soil texture was determined with the pipette method according to Gee & Bauder (1986).

Soil acidity and mineral N concentrations

Three weeks after the application of synthetic cattle urine, four soil samples (2 cm diameter × 5 cm depth) were collected in each subplot. The soils were sieved, roots removed and soil pH measured potentiometrically in a dried (60 °C) aliquote suspended in 0.01 M CaCl2 at a soil:extractant ratio of 1 : 2.

Ammonium (NH_4^+) and nitrate (NO_3^-) were extracted from 10 g fresh soil with 100 mL 1M KCl in an overhead shaker (1.5 h). Extracts were filtered (0790½, Whatman International, Maidstone, UK) and NH_4^+ concentrations were measured colorimetrically by automated flow injection analysis (Perkin Elmer UV/VIS Spectrometer Lambda 2S, Waltham, MA, USA). Nitrate was determined colorimetrically at 210 nm (Varian Cary 50, Palo Alto CA, USA) as difference in absorbance between nonreduced and reduced (using H_2SO_4 and copperized zinc) extracts (Navone, 1964).

Potential nitrification and denitrification

Potential nitrification (PN) was determined by the shaken slurry method (Hart *et al.*, 1994). Briefly, 10 g sieved fresh soil was suspended in 90 mL 1 mM phosphate buffer adjusted to pH 7.0. Ammonium sulphate (140 mg N kg^{-1} soil) was added and the slurry incubated at 25 °C on an orbital shaker. Aliquots of 10 mL were taken after 1, 4, 18 and 22 h. These aliquots were immediately mixed with 15 mL 2.5 M KCl to stop nitrification, centrifuged, and the supernatant analysed for NO_3^- as described above. Potential nitrification rates were calculated by linear regression of NO_3^- concentration against time.

Denitrifying enzyme activity (DEA), which shows the denitrification potential under excess substrate availability, was determined by the application of the acetylene inhibition assay (Smith & Tiedje, 1979; Patra *et al.*, 2005). Fresh sieved soil samples equivalent to 5 g dry weight were placed in 125 mL plasma flasks and the headspace replaced by a 90 : 10 mixture of helium:acetylene. The flasks were incubated at 26 °C and, after 1 h, an aqueous solution containing KNO_3, glucose and glutamic acid was added. N_2O concentrations in the headspace were analysed after 60, 90 and 120 min as described above. N_2O production per unit time (DEA) was estimated by linear regression.

Tree aboveground biomass

Tree aboveground biomass in each plot was calculated using allometric relations depending on stem diameter at breast height, tree height (Kaufmann, 2001) and basal area per ground area. The diameter of all trees was measured in two areas, 25–100 m^2 in size in stands up to 30 years old. In the older afforestations, trees were measured in a single large area of 250–600 m^2 to account for the bigger size and lower density of trees found there. In addition, the heights of 5–10 single trees per area were measured.

Statistical analysis

We analysed our data by fitting mixed-effects models by maximum likelihood (ASReml 3.0, VSN International, UK; Gilmour *et al.*, 2009). The model included the sequential fixed effects altitude (elevation in m a.s.l.), land use (forest vs. meadow), forest stand age, fertilization and the interactions of fertilization with land use and stand age. The effect of stand age was fitted as a log-linear contrast [1 df, testing for effects of log(age)] followed by a term testing for the deviation from log-linearity (3 df, age fitted as categorical term). The significance of the fixed effects was determined using Wald statistics. Reflecting the structure of the experiment, the model included the nested random effects plot, subplot and static chamber. Altitude and stand age were partly confounded in our study, with higher average forest stand age at the bottom of the slope, and younger forest patches dominating the top of the slope. We therefore fitted a second model in which the terms for altitude and stand age were interchanged; testing for effects of age after accounting for altitude underestimates the age effect, whereas age effects potentially include an altitude component when fitted first. Effects with $P < 0.05$ were considered statistically significant, effects with $0.05 < P < 0.1$ as marginally significant.

Results

Soil bulk density, porosity and water content

Average bulk density of the soils (0–10 cm) showed no consistent trend with land use and stand age. The

densities varied between 0.7 and 0.9 g cm^{-3}, with the highest values in the 40 years old afforestations (0.91 g cm^{-3}) and the lowest ones in the old forest (0.70 g cm^{-3}) (Table 1). These findings were confirmed by measuring an additional 65 soil cores sampled across the whole site; these did not show a statistically significant effect of stand age or land use on bulk density (data not shown). In accordance, soil porosities were in a rather narrow range (63–69%) and also did not depend on land use or stand age (Table 1).

Microclimate greatly differed between the two land-use types. During the growing season, surface soils of the forest stands were on average 5 °C cooler than the pasture soils, which exceeds the temperature lapse rate across 250 m in altitude of 1.5 °C. Soil moisture varied within the pasture, but this variation was not related to altitude. In fact, pasture soils at the top and the bottom of the slope had approximately equal soil water contents of 0.40 m^3 m^{-3} when averaged over the six sampling dates (Fig. 2c). A general trend, however, was that soil moisture significantly decreased with stand age on all except one date, with variable levels of significance ($P < 0.05$ to $P < 0.001$). The measurement period encompassed a wide range of climatic conditions resulting with rather dry (14–26% volumetric water content; July 17, 2010) and wet soils (24–57%; October 3, 2010). Forest soils were drier than pasture soils on all dates. Reflecting soil moisture, water-filled pore space (WFPS) also decreased along the chronosequence (Fig. 3).

Soil methane uptake

Soil CH_4 uptake was higher under forest than under pasture ($P < 0.001$ for effects of land use). Soil CH_4 uptake significantly increased with stand age, with the log-linear component ($P < 0.001$) explaining twice as much variance as the term testing for deviations from log-linearity ($P < 0.05$). Stand age explained less variance when fitted after accounting for altitude ($P = 0.01$ for log(age) and $P = 0.08$ for the deviation from log-linearity). Reflecting the partially confounding influence of age and altitude, the effect of altitude was significant at $P < 0.001$ when fitted before age, but explained ~8 times less variance and was at the border to significance ($P = 0.05$) when fitted after age.

Water-filled pore space was significantly negatively related to soil CH_4 uptake ($P < 0.001$; Fig. 3), explaining more than 70% of the variance accounted for by the fixed effects contained in the model. Effects of log(age) explained only half as much variance and were less significant ($P < 0.05$) when fitted after WFPS, suggesting that at least part of the observed age effect was due to altered soil moisture. Soil NH_4^+ and NO_3^- (fitted as log($[NH_4^+]$) and $\sqrt{}[NO_3^-]$) did not explain significant fractions of the variation in CH_4 fluxes.

Cattle urine addition exerted only little effect on CH_4 oxidation; when data for the different sampling dates were tested individually, a decrease in soil CH_4 uptake of 20% was found 1 day after fertilizer addition ($P = 0.02$). Averaged over all sampling dates, effects of cattle urine addition were no longer statistically significant (−11%, n.s.).

Interestingly, the CH_4 uptake of sieved soils incubated in the laboratory did not reveal any systematic effect of age (Fig. 4), but effects of soil moisture remained significant at $P < 0.001$.

Soil N_2O emissions and N cycling

In the absence of cattle urine, N_2O fluxes did not change with age. Cattle urine increased N_2O emissions from soils in the younger forest stands. This resulted in a significant overall effects of log(age) ($P < 0.001$) and a significant interaction between log(age) and cattle urine application ($P < 0.05$, respectively, Fig. 2b). Soil extractable NH_4^+ increased with stand age ($P < 0.05$), whereas NO_3^- did not show such an effect (Fig. 5a, b). Potential nitrification did not depend on stand age, but increased with cattle urine addition ($P < 0.01$) (Fig. 5c). Denitrification enzyme activity did not respond either to stand age or to cattle urine addition (Fig. 5d). Soil acidity

Table 1 Soil properties and aboveground tree biomass in the pasture and the different afforestations with (standard errors) representing the different plots per age

Land use	pH ($CaCl_2$)		Bulk density (g cm^{-3})		Porosity (%)		Clay content (%)		Tree biomass (t ha^{-1})	
Pasture	4.9	(0.1)	0.83	(0.04)	65	(1.4)	55	(1)	-	-
Afforestation 25 years	4.8	-	0.73	-	69	-	57	-	157	-
Afforestation 30 years	4.9	(0.3)	0.79	(0.06)	67	(1.5)	55	(4)	140	(3)
Afforestation 40 years	4.2	(0.0)	0.91	(0.05)	63	(1.4)	36	(12)	277	(38)
Afforestation 45 years	3.9	-	0.76	-	68	-	51	-	263	-
Afforestation 120 years	4.8	-	0.70	-	69	-	49	-	579	-

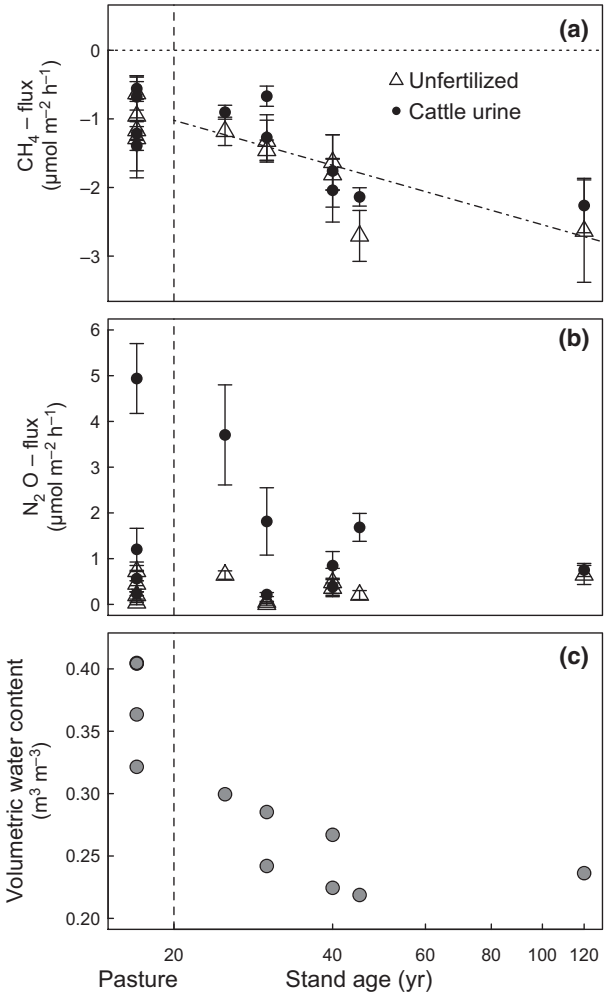

Fig. 2 Fluxes of CH_4 (a) and N_2O (b) in dependence of forest stand age and cattle urine application (negative values indicate uptake from the atmosphere). Error bars are standard errors based on $n = 4$ subplots per forest or pasture plot. Cattle urine addition did not affect volumetric soil water content (c), so that data of fertilized and unfertilized subplots were combined.

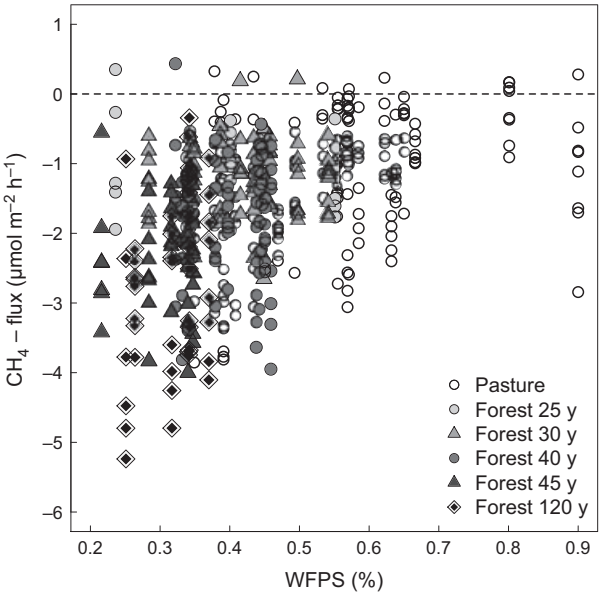

Fig. 3 Soil CH_4 fluxes in dependence of water-filled pore space (WFPS) and stand age (negative values indicate uptake from the atmosphere).

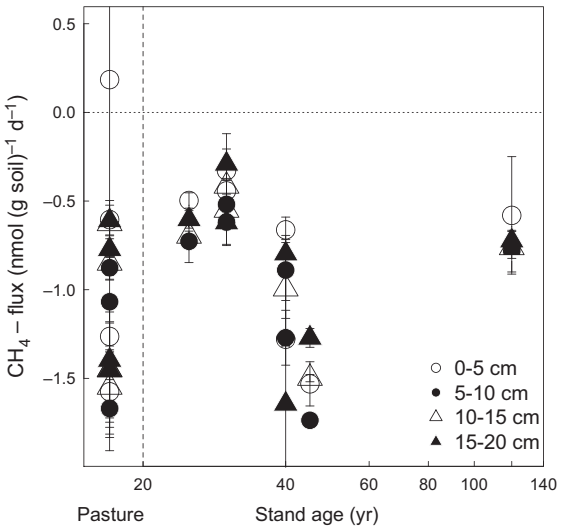

Fig. 4 CH_4 fluxes of sieved soils incubated in the laboratory, in dependence of stand age and soil depth (negative values indicate uptake from the atmosphere). Error bars show standard errors of means.

generally decreased with stand age, but the oldest stand had pH similar to the youngest stand; this resulted in no effect of log(age), but a significant deviation from linearity ($P < 0.001$).

Discussion

Our results show that CH_4 oxidation in subalpine soils increased by a factor of two to three after conversion from pasture to forest. CH_4 oxidation increased with stand age on all sampling dates, spanning a wide range of climatic conditions, emphasizing that this effect is robust.

In contrast to CH_4 fluxes, N_2O emissions showed no similar change with stand age, at least as long as no cattle urine was added (Fig. 2b). The main driver of N_2O emissions was the mineral N status of the soils, but particularly concentrations of NO_3^- were not related to stand age. The primary objective of the N_2O flux measurements was not to assess N_2O fluxes in detail (which would require far more measurements), but to obtain an indicator of the ecosystem's N status and its dependency on age and fertilizer application.

In the 120-year-old subalpine forest, soil CH_4 oxidation had reached rates comparable to the range

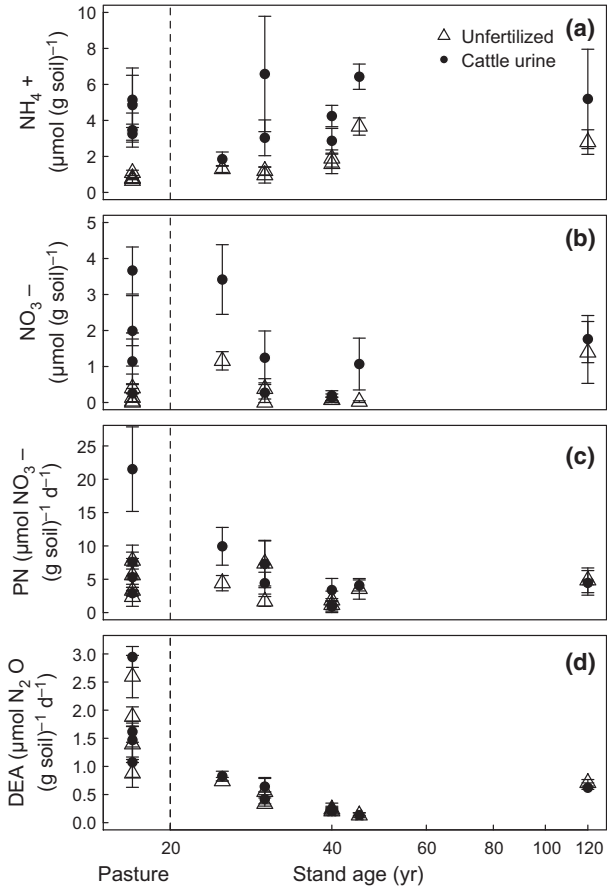

Fig. 5 Soil extractable NH_4^+ (a) and NO_3^- (b), potential nitrification rates (PN) (c) and denitrification enzyme activity (DEA) (d) in dependence of forest stand age and cattle urine application. All data refer to the 5 cm of mineral soil. Error bars represent standard errors.

published for temperate coniferous forests (Smith *et al.*, 2000; Jang *et al.*, 2006; Peichl *et al.*, 2010), although much higher rates have been reported for some forests (Ishizuka *et al.*, 2000; Price *et al.*, 2004). However, it remains unclear whether further increases in CH_4 oxidation can be expected in the future when stand age exceeds 120 years.

Stand age and altitude were not orthogonal in our study. The oldest and the 45 years old forest were at the lower end of the slope (1450 m a.s.l.), whereas the youngest (25 years old) stand was at the upper end at 1700 m a.s.l. CH_4 uptake is relatively insensitive to temperature (Smith *et al.*, 2003), and therefore not likely to be affected by the relatively short altitudinal gradient of 250 m. Nevertheless, vegetation period, plant growth and the biological activity might be higher at the lower end of the slope and effects of altitude could therefore be confounded with effects of stand age. We argue, however, that this is unlikely in our study, for several reasons. First, and most importantly, the effect of stand age remained statistically significant after adjusting for altitude. Second, the effect of altitude was not statistically significant in the reference grassland ($P = 0.09$), although there was a slight trend towards increased CH_4 oxidation rates at lower elevation. Third, if the effect of altitude was to increase productivity and the length of the growing season, then one might argue that the forest stands at the bottom of the slope are even older on a biomass or 'degree-days' scale. In this case, altitude would increase the 'effective age' of the older stands more than the one of the younger stands, and thus do not alter the conclusions. Indeed, when we analysed CH_4 oxidation as function of stand biomass (which is a proxy for biomass), we obtained similar results ($P < 0.001$).

Stand-age effects found in the few afforestation studies available to date are ambiguous. Whereas the majority of the studies observed a slow increase of CH_4 uptake after tree establishment (Priemé *et al.*, 1997; Singh *et al.*, 2007; McNamara *et al.*, 2008; Peichl *et al.*, 2010), some found no age effect (Ball *et al.*, 2007), or an age effect which depended on tree species (Christiansen & Gundersen, 2011). However, the mechanisms driving this change remain uncertain. One possibility might be that the populations of methanotrophic bacteria require decades to increase due to slow growth rates under atmospheric CH_4 concentrations (Priemé *et al.*, 1996; King, 1997; Menyailo *et al.*, 2008). In addition, the change from herbaceous to tree cover may induce shifts in methanotrophic community structure. In New Zealand, Singh *et al.* (2009) related higher soil CH_4 uptake in pine afforestation compared with pastures to a higher activity of type II methanotrophs which are thought to oxidize atmospheric CH_4 in soils (Knief *et al.*, 2006). We did not measure methanotrophic community structure; however, the systematic effect of stand age was lost when CH_4 oxidation rates were measured on sieved soils, i.e. when diffusive limitations by soil horizons were eliminated. This suggests that the potential to oxidize CH_4 was similar at all sites, independent of age, although our comparison of incubation and field measurements clearly has some limitations. In particular, laboratory experiments only reflect the oxidation potential of the incubated soil layer, excluding processes lower in the soil column, e.g. methanogenesis. Discrepancies between laboratory incubations and *in situ* measurements were also reported, for example by (Reay *et al.*, 2005), who measured considerable CH_4 uptake in sieved grassland soils incubated in the laboratory while the same soils were net sources of CH_4 under field conditions (Nedwell *et al.*, 2003). We argue that different diffusive limitations are the most likely explanation for the discrepancy between CH_4 uptake in laboratory incubations and *in situ*. Soil gas diffusivity is

controlled by pore network structure and water-filled pore space. In our study, CH_4 uptake decreased with water-filled pores space, a phenomenon commonly found (Dörr *et al.*, 1993; Ball *et al.*, 1997a; Bowden *et al.*, 1998). Soil bulk density and porosity varied only little (60–70%) among plots, with no systematic effect of stand age. Soil bulk density was fairly low even in the pasture plots, mainly because the cattle moved on specific tracks, leaving the major part of the pastures unaffected by trampling (Hiltbrunner *et al.*, 2012).

Why did soil moisture decrease with forest stand age? Evapotranspiration and interception often increase with forest age (Farley *et al.*, 2005). Moreover, the organic layer under spruce trees shelters the underlying mineral soil from rainfall (Borken & Beese, 2006). In our study, a gradual accumulation of the organic layer, reaching a thickness of 4–10 cm in the two oldest stands, might have contributed to drier mineral soils in the older forest plots. In conjunction with increased water retention by the forest stand, this might have promoted CH_4 diffusion into soils, which in turn enhanced CH_4 uptake with forest development.

High NH_4^+ concentrations can inhibit soil CH_4 oxidation in many ecosystems (Gulledge *et al.*, 1997; King & Schnell, 1994; Le Mer & Roger, 2001; Smith *et al.*, 2000; Steudler *et al.*, 1989). In our study, soil extractable NH_4^+ increased with stand age, with no evidence of an inhibition of CH_4 uptake. Similarly, Tate *et al.* (2007) also did not find a significant relationship between extractable NH_4^+ and soil CH_4 oxidation in a land-use change study in New Zealand, despite relatively high soil NH_4^+ concentrations. The cattle urine application in our study also did not substantially suppress soil CH_4 uptake, despite relatively large amounts added and resulting in increased contents of extractable NH_4^+ and increased associated N_2O emissions in the following 2 months. However, the fertilization effects were largest in the younger stands, raising the possibility that rapid N uptake by more N-limited old forest stands and their soils protected methanotrophs against effects of NH_4^+.

CH_4 oxidation often decreases with soils acidification, either due to direct effects of soil pH, or due to reduced nitrification rates and therefore increased soil NH_4^+ concentrations (Weslien *et al.*, 2009; Stiehl-Braun *et al.*, 2011). Although soil pH differed between forest plots in our study, these changes did not explain the patterns observed in soil CH_4 uptake. Furthermore, soil pH changes spanned only one single pH unit.

Currently, forest cover is increasing rapidly in the European Alps. Our data can be combined with estimates of land-use change to arrive at an educated guess of the order of magnitude by which soil CH_4 uptake may increase as consequence of land abandonment. We base our calculation on Switzerland, but expect similar changes in other European alpine areas. Forest cover increased by more than 90 000 ha between 1984 and 2005 in the Swiss Alps (Brändli, 2010), which is equivalent to as much as 8% per decade. The Swiss alpine forests are dominated by conifers, covering 75–85% of the total forested area, with Norway spruce being by far the most abundant species (Brändli, 2010). We assume that (i) the investigated forest stands are reasonably representative of the new forest area, (ii) our flux measurements are a good estimate of soil CH_4 uptake for the snow-free period (May to October) and (iii) the difference in soil CH_4 uptake between pasture and 45 years old stands reflects the anticipated changes (Δ = 1.0–1.5 μmol CH_4 m^{-2} h^{-1}). Combining these data yields an increase in soil CH_4 uptake in the order of ~0.5–0.8 kg CH_4–C ha^{-1} yr^{-1} or ~50–70 t CH_4–C for the entire 90 000 ha area. Soil CH_4 uptake has been estimated at ~6000 t CH_4-C yr^{-1} for Switzerland (Minonzio *et al.*, 1998). However, this figure is associated with a large uncertainty (minimum ~1000 t, maximum ~18 000 t CH_4 yr^{-1}) mainly due to a lack of data for forest soil CH_4 uptake. Our data thus suggest that the ongoing forest expansion in alpine areas increases the Swiss soil CH_4 sink by up to a few percent per decade.

In summary, our study shows increases in soil CH_4 uptake by a factor of two to three after conversion from subalpine pasture to forest. Our data indicate that the most likely reason for this change was shifts in the soil moisture balance due to increased interception and higher evapotranspiration in older forest stands. As a consequence, water-filled pore space decreased and the diffusion of atmospheric CH_4 into soils was facilitated. This mechanism contrasts alternative mechanisms suggested, including altered soil N status, altered soil structure or shifts in the methanotrophic community structure (Priemé *et al.*, 1997; Singh *et al.*, 2007; Christiansen & Gundersen, 2011).

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References

Ball BC, Dobbie KE, Parker JP, Smith KA (1997a) The influence of gas transport and porosity on methane oxidation in soils. *Journal of Geophysical Research-Atmospheres*, **102**, 23301–23308.

Ball BC, Smith KA, Klemedtsson L *et al.* (1997b) The influence of soil gas transport properties on methane oxidation in a selection of northern European soils. *Journal of Geophysical Research-Atmospheres*, **102**, 23309–23317.

Ball T, Smith KA, Moncrieff JB (2007) Effect of stand age on greenhouse gas fluxes from a Sitka spruce (Picea sitchensis (Bong.) Carr.) chronosequence on a peaty gley soil. *Global Change Biology*, **13**, 2128–2142.

Blake GR, Hartge KH (1986) Particle Density. In: *Methods of Soil Analysis, Part I. Physical and Mineralogical Methods* (ed. Klute A), pp. 363–375. Amer. Soc. Agron. and Soil Sci. Soc. Amer., Madison (Wisconsin), USA.

Bodelier PLE, Laanbroek HJ (2004) Nitrogen as a regulatory factor of methane oxidation in soils and sediments. *Fems Microbiology Ecology*, **47**, 265–277.

Boeckx P, Van Cleemput O (2001) Estimates of N_2O and CH_4 fluxes from agricultural lands in various regions in Europe. *Nutrient Cycling in Agroecosystems*, **60**, 35–47.

Boone DR, Whitman WB, Rouviére P (1993) Diversity and taxonomy of methanogens. In: *Methanogenesis* (ed. Ferry JG), pp. 35–80. Chapman and Hall, New York.

Borken W, Beese F (2006) Methane and nitrous oxide fluxes of soils in pure and mixed stands of European beech and Norway spruce. *European Journal of Soil Science*, **57**, 617–625.

Bowden RD, Newkirk KM, Rullo GM (1998) Carbon dioxide and methane fluxes by a forest soil under laboratory-controlled moisture and temperature conditions. *Soil Biology & Biochemistry*, **30**, 1591–1597.

Brändli U-B (2010) *Schweizerisches Landesforstinventar. Ergebnisse der dritten Erhebung 2004–2006*. Eidgenössische Forschungsanstalt für Wald, Schnee und Landschaft WSL., Birmensdorf, Bundesamt für Umwelt, BAFU, Bern.

Christiansen JR, Gundersen P (2011) Stand age and tree species affect N_2O and CH_4 exchange from afforested soils. *Biogeosciences*, **8**, 2535–2546.

Dörr H, Katruff L, Levin I (1993) Soil texture parameterization of the methane uptake in aerated soils. *Chemosphere*, **26**, 697–713.

FAO (2001) *Global forest resources assessment 2000*. Main report, FAO Forestry Paper 140, Rome, Italy.

Farley KA, Jobbagy EG, Jackson RB (2005) Effects of afforestation on water yield: a global synthesis with implications for policy. *Global Change Biology*, **11**, 1565–1576.

Fraser PM, Cameron KC, Sherlock RR (1994) Lysimeter study of the fate of nitrogen in animal urine returns to irrigated pasture. *European Journal of Soil Science*, **45**, 439–447.

Gee GW, Bauder JW (1986) Particle size analysis. In: *Methods of Soil Analysis. Part 1. Physical and Mineralogical Methods* (ed. Klute A), pp. 386–411. Am. Soc. Agron, Madison, WI.

Gilmour AR, Gogel BJ, Cullis BR, Thompson R (2009) *ASReml User Guide Release 3.0*. VSN International Ltd, Hemel Hempstead, UK.

Gulledge J, Doyle AP, Schimel JP (1997) Different NH_4^+-inhibition patterns of soil CH_4 consumption: a result of distinct CH_4-oxidizer populations across sites? *Soil Biology & Biochemistry*, **29**, 13–21.

Hart SC, Stark JM, Davidson EA, Firestone MK (1994) Nitrogen mineralisation, immobilisation and nitrification. In: *Methods of Soil Analysis, Part 2. Microbilological and Biochemical Properties-SSSA* (ed. Weaver RW *et al.*), pp. 985–1018. Soil Sci. Soc. Am., Madison, WI, USA.

Hiltbrunner D, Schulze S, Hagedorn F, Schmidt MWI, Zimmmermann S (2012) Cattle trampling alters soil properties and changes soil microbial communities in a Swiss sub-alpine pasture. *Geoderma*, **170**, 369–377.

Hütsch BW (1998) Methane oxidation in arable soil as inhibited by ammonium, nitrite, and organic manure with respect to soil pH. *Biology and Fertility of Soils*, **28**, 27–35.

Hütsch BW, Webster CP, Powlson DS (1994) Methane oxidation in soil as affected by land-use, soil-pH and N-fertilization. *Soil Biology & Biochemistry*, **26**, 1613–1622.

IPCC (2007). In: *Climate Change 2007: The Physical Science Basis Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change* (eds Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL), pp. 996. Cambridge University Press, Cambridge, United Kingdom and New York, USA.

Ishizuka S, Sakata T, Ishizuka K (2000) Methane oxidation in Japanese forest soils. *Soil Biology & Biochemistry*, **32**, 769–777.

Jang I, Lee S, Hong J-H, Kang H (2006) Methane oxidation rates in forest soils and their controlling variables: a review and a case study in Korea. *Ecological Research*, **21**, 849–854.

Jassal RS, Black TA, Roy R, Ethier G (2011) Effect of nitrogen fertilization on soil CH_4 and N_2O fluxes, and soil and bole respiration. *Geoderma*, **162**, 182–186.

Kaufmann E (2001) Estimation of standing timber, growth and cut. In: *Swiss National Forest Inventory: Methods and Models of the Second Assessment* (eds. Brassel P, Lischke H), pp. 162–196. Swiss Federal Research Institute WSL, Birmensdorf.

King GM (1997) Responses of atmospheric methane consumption by soils to global climate change. *Global Change Biology*, **3**, 351–362.

King GM, Schnell S (1994) Ammonium and nitrite inhibition of methane oxidation by methylobacter-albus BG8 and methylosinus-trichosporium OB3b at low methane concentrations. *Applied and Environmental Microbiology*, **60**, 3508–3513.

Knief C, Kolb S, Bodelier PLE, Lipski A, Dunfield PF (2006) The active methanotrophic community in hydromorphic soils changes in response to changing methane concentration. *Environmental Microbiology*, **8**, 321–333.

McNamara NP, Black HIJ, Piearce TG, Reay DS, Ineson P (2008) The influence of afforestation and tree species on soil methane fluxes from shallow organic soils at the UK Gisburn Forest Experiment. *Soil Use and Management*, **24**, 1–7.

Menyailo OV, Hungate BA, Abraham W-R, Conrad R (2008) Changing land use reduces soil CH_4 uptake by altering biomass and activity but not composition of high-affinity methanotrophs. *Global Change Biology*, **14**, 2405–2419.

Le Mer J, Roger P (2001) Production, oxidation, emission and consumption of methane by soils: a review. *European Journal of Soil Biology*, **37**, 25–50.

Minonzio G, Grub A, Fuhrer J (1998) *Methan-Emissionen der schweizerischen Landwirtschaft*. Schriftenreihe Umwelt Nr. 298. Bundesamt fu r Umwelt, Wald und Landschaft (BUWAL), Bern.

Navone R (1964) Proposed method for nitrate in potable waters. *Journal of American Water Works Association*, **56**, 781–783.

Nedwell DB, Murrell JC, Ineson P, Reay DS, Radajewski S, McNamara N, Morris S (2003) Microbiological basis of land use impact on the soil methane sink: Molecular and functional analysis. In: *Genes in the Environment* (eds Hails RS, Beringer JE, Godfray HC J), pp. 15–166. Blackwell, London.

Patra AK, Abbadie L, Clays-Josserand A *et al.* (2005) Effects of grazing on microbial functional groups involved in soil N dynamics. *Ecological Monographs*, **75**, 65–80.

Peichl M, Arain MA, Ullah S, Moore TR (2010) Carbon dioxide, methane, and nitrous oxide exchanges in an age-sequence of temperate pine forests. *Global Change Biology*, **16**, 2198–2212.

Price SJ, Sherlock RR, Kelliher FM, McSeveny TM, Tate KR, Condron LM (2004) Pristine New Zealand forest soil is a strong methane sink. *Global Change Biology*, **10**, 16–26.

Priemé A, Sitaula JIB, Klemedtsson AK, Bakken LR (1996) Extraction of methane-oxidizing bacteria from soil particles. *Fems Microbiology Ecology*, **21**, 59–68.

Priemé A, Christensen S, Dobbie KE, Smith KA (1997) Slow increase in rate of methane oxidation in soils with time following land use change from arable agriculture to woodland. *Soil Biology & Biochemistry*, **29**, 1269–1273.

Reay DS, Nedwell DB, McNamara N, Ineson P (2005) Effect of tree species on methane and ammonium oxidation capacity in forest soils. *Soil Biology & Biochemistry*, **37**, 719–730.

Regina K, Pihlatie M, Esala M, Alakukku L (2007) Methane fluxes on boreal arable soils. *Agriculture Ecosystems & Environment*, **119**, 346–352.

Singh BK, Tate KR, Kolipaka G, Hedley CB, Macdonald CA, Millard P, Murrell JC (2007) Effect of afforestation and reforestation of pastures on the activity and population dynamics of methanotrophic bacteria. *Applied and Environmental Microbiology*, **73**, 5153–5161.

Singh BK, Tate KR, Ross DJ *et al.* (2009) Soil methane oxidation and methanotroph responses to afforestation of pastures with Pinus radiata stands. *Soil Biology & Biochemistry*, **41**, 2196–2205.

Smith MS, Tiedje JM (1979) Phases of denitrification following oxygen depletion in soil. *Soil Biology & Biochemistry*, **11**, 261–267.

Smith KA, Dobbie KE, Ball BC *et al.* (2000) Oxidation of atmospheric methane in Northern European soils, comparison with other ecosystems, and uncertainties in the global terrestrial sink. *Global Change Biology*, **6**, 791–803.

Smith KA, Ball T, Conen F, Dobbie KE, Massheder J, Rey A (2003) Exchange of greenhouse gases between soil and atmosphere: interactions of soil physical factors and biological processes. *European Journal of Soil Science*, **54**, 779–791.

Steudler PA, Bowden RD, Melillo JM, Aber JD (1989) Influence of nitrogen-fertilization on methane uptake in temperate forest soils. *Nature*, **341**, 314–316.

Stiehl-Braun PA, Powlson DS, Poulton PR, Nildaus PA (2011) Effects of N fertilizers and liming on the micro-scale distribution of soil methane assimilation in the long-term Park Grass experiment at Rothamsted. *Soil Biology & Biochemistry*, **43**, 1034–1041.

Tate KR, Ross DJ, Saggar S, Hedley CB, Dando J, Singh BK, Lambie SM (2007) Methane uptake in soils from Pinus radiata plantations, a reverting shrubland and adjacent pastures: effects of land-use change, and soil texture, water and mineral nitrogen. *Soil Biology & Biochemistry*, **39**, 1437–1449.

Weslien P, Klemedtsson AK, Borjesson G, Klemedtsson L (2009) Strong pH influence on N_2O and CH_4 fluxes from forested organic soils. *European Journal of Soil Science*, **60**, 311–320.

Whalen SC (2000) Influence of N and non-N salts on atmospheric methane oxidation by upland boreal forest and tundra soils. *Biology and Fertility of Soils*, **31**, 279–287.

Willison TW, Webster CP, Goulding KWT, Powlson DS (1995) Methane oxidation in temperate soils – effects of land-use and the chemical form of nitrogen-fertilizer. *Chemosphere*, **30**, 539–546.