This is a postprint of an article published in

enyailo, O.V., Abraham, W.-R., Conrad, R.

Tree species affect atmospheric CH4 oxidation without altering community composition of soil methanotrophs

(2010) Soil Biology and Biochemistry, 42 (1), pp. 101-107.

1 *Soil Biology & Biochemistry*

2 # Tree species affect atmospheric CH_4 oxidation without altering community
3 composition of soil methanotrophs

4

5 Running title: Tree species and soil methanotrophs

6

7 Oleg V. Menyailo[1,2], Wolf-Rainer Abraham[3], and Ralf Conrad[2]

8 [1]V.N.Sukachev Institute of Forest SB RAS, Krasnoyarsk 660036, Russia

9 [2]Max Plank Institute for Terrestrial Microbiology, Karl-von-Frisch-Strasse, Marburg 35043,
10 Germany

11 [3]Helmholtz Center for Infection Research, Inhoffenstrasse 7, D-38124 Braunschweig, Germany

12

13 *Corresponding author:* Dr. Oleg Menyailo, Institute of Forest SB RAS, Krasnoyarsk 660036,
14 Russia, Fax: 7 3912 433686, Email: menyailo@hotmail.com

15

16

17

18

19 *Key words*: Atmospheric methane, High affinity methanotrophs, ^{13}C-labeling, Microbial diversity,
20 Net N mineralization, Net nitrification, PLFA, Tree species

21

22

23

24

25 **Abstract**

26 Plant species exert strong effects on ecosystem functions and one of the emerging, and difficult
27 to test hypotheses, is that plants alter soil functions through changing the community structure of
28 soil microorganisms. We tested the hypothesis for atmospheric CH_4 oxidation by using soil samples
29 from a Siberian afforestation experiment and exposing them to ^{13}C-CH_4. We determined the activity
30 of the soil methanotrophs under different tree species at three levels of initial CH_4 concentration (30,
31 200 and 1000 ppm) thus distinguishing the activities of low- and high-affinity methanotrophs. Half
32 of the samples were incubated with ^{13}C-enriched CH_4 (99.9%) and half with ^{12}C-CH_4. This allowed
33 an estimation of the amount of ^{13}C incorporated into individual PLFAs and determination of PLFAs
34 of methanotrophs involved in CH_4 oxidation at the different CH_4 concentrations. Tree species
35 strongly altered the activity of atmospheric CH_4 oxidation without appearing to change the
36 composition of high-affinity methanotrophs as evidenced by PLFA ^{13}C labeling. The low diversity
37 of atmospheric CH_4 oxidizers, presumably belonging to the UCSα group, may explain the lack of
38 tree species effects on the composition of soil methanotrophs. We submit that the observed tree
39 species effects on atmospheric CH_4 oxidation indicate an effect on biomass or cell-specific activities
40 rather than by a community change and this may be related to the impact of the tree species on soil
41 N cycling.

42
43

44

45

46

47

48

49

50

51

52

53 ## 1. Introduction

54 Forest soils are important sink for atmospheric CH_4, a greenhouse gas contributing roughly 20% to
55 the observed global warming (IPCC, 2007). While tree species strongly influence the sink strength
56 (Borken et al., 2003; Menyailo and Hungate, 2003; Reay et al., 2004; Borken and Beese, 2006), the
57 mechanisms underlying the tree species effects are poorly understood. Tree species having different
58 canopy cover, rooting depth and density, alter soil temperature, moisture and pH (Angers and Caron,
59 1998; Hooper et al., 2000); tree species also differ in litter quality and quantity. Generally, tree
60 species can also affect the structure of microbial community including microbial biomass (Priha and
61 Smolander, 1997; Grayston and Prescott, 2005; Lejon et al., 2005; Menyailo, 2007), microbial
62 species composition (Lejon et al., 2005; Carney and Matson, 2006; Bremer et al., 2007; Nugroho et
63 al., 2007) and diversity (Zak et al., 2003; Hackl et al., 2004; Bremer et al., 2007). However, the
64 importance of changes in the composition and diversity of soil methanotrophs for further mediating
65 tree species effects on the consumption of atmospheric CH_4 has never been studied.

66 Molecular studies in forest soils have revealed that atmospheric CH_4 is oxidized by
67 methanotrophs consisting of as yet uncultivated groups, including upland soil cluster α (USCα) and
68 upland soil cluster γ (USCγ) (e.g. Knief et al., 2003, 2006; Kolb et al., 2005; Lau et al., 2007;
69 McDonald et al., 2008). Members of USCα and USCγ are presumed to be specialized oligotrophs
70 adapted to live solely on atmospheric concentration of CH_4. Until recently, all cultured
71 methanotrophs were assumed to be incapable of surviving on low concentrations of atmospheric
72 CH_4 because of their low affinity for methane. However, recent work by Baani and Liesack (2008)
73 has provided evidence that *Methylocystis sp.* strain SC2 possesses two types of the particulate
74 methane monooxygenase, a key enzyme in CH_4 oxidation. The two forms of the enzyme have
75 different affinities for CH_4, allowing *Methylocystis spp.* to survive on atmospheric CH_4 for three
76 months at 10-100 ppm of CH_4 (Knief and Dunfield, 2005), while other cultivated species can only

77 grow at 500-1000 ppm. It has also been suggested that *Methylocystis spp*. is the main oxidizer of
78 atmospheric CH_4 in hydromorphic soils (Baani and Liesack, 2008), where the growth of the
79 methanotroph can be supported by CH_4 formed in high concentrations in waterlogged anaerobic
80 zones; in contrast, *Methylocystis spp*. start to oxidize CH_4 from the atmosphere when soil is
81 temporally dry and aerobic. Whether *Methylocystis spp*. are active in forest upland soils is still
82 questionable, and the participation of uncultured members of USCα and USCγ cannot be excluded.

83 A combination of phospholipid fatty acid (PLFA) analysis and stable isotope probing (SIP) has
84 been used recently to identify methanotrophs active at different CH_4 concentrations (Bull et al.,
85 2000; Crossman et al., 2005; Maxfield et al., 2006; Knief et al., 2003, 2006). While the SIP-PLFA
86 technique does not have the resolution of molecular techniques and cannot identify organisms to
87 species level, it can readily distinguish methanotrophs at a group level, such as Type I and Type II
88 (Bodelier et al., 2009), which differ in intracytoplasmic membrane structure, carbon assimilation
89 patterns, phylogeny and carbon chain length of membrane phospholipid fatty acids (Hanson and
90 Hanson, 1996). Furthermore, SIP-PLFA allows conclusions to be drawn as to whether the composition
91 of active soil methanotrophs is different between different sites. Here, we applied the SIP-PLFA
92 technique to understand whether tree species effects on CH_4 oxidation rates may be explained by
93 changes in the community structure of high affinity methanotrophs.

94 Most experiments on the effects of plants on soils have been carried out using agricultural or
95 herbaceous plants with experiments testing the effects of individual tree species being more difficult
96 to establish and maintain over a long period. There are not many long-term afforestation
97 experiments with different tree species around the world (Priha and Smolander, 1997) and one of
98 them is the Siberian afforestation experiment, where six common boreal tree species have been
99 grown for the past 35 years (Menyailo et al., 2002a). Since the experiment began with an initially
100 uniform soil, and all plots have been exposed to the same climatic conditions, differences in soil
101 properties can be attributed to the effects of tree species. The aim of this work was to determine
102 whether different tree species affect the oxidation rates of atmospheric CH_4 and whether these
103 effects are due to changes in the composition of high-affinity methanotrophs.

104

105

106 ## 2. Materials and methods

107 *2.1. Siberian afforestation experiment*

108 The research plots are located 50 km northwest from Krasnoyarsk and were established by the
109 Laboratory of Soil Science of the Institute of Forest, Siberian Branch of the Russian Academy of
110 Sciences. The experimental plots were established on grassland. In 1971-1972, 2-3 y old seedlings
111 of spruce (*Picea abies*), birch (*Betula pendula*), Scots pine (*Pinus sylvestris*), aspen (*Populus*
112 *tremula*), larch (*Larix sibirica*) and Arolla pine (*Pinus cembra*) were sown into individual plots,
113 each occupying 2400 m^2 (Menyailo et al., 2002a). The region is characterized by continental
114 climatic conditions with average rainfall of 500 mm $year^{-1}$, average daily summer temperature of
115 20°C (at 12:00) and soil temperature at 20 cm depth in winter is −4 to −14 ◦C and 10 to 12 ◦C in
116 summer.The soil is the grey forest type according to the Russian Soil Classification System and
117 Greyzem according to FAO. In July 2005, each plot was sub-divided into three parts: A, B and C
118 and, from each sub-plot, two trees were randomly chosen and four samples of mineral soil (0-10 cm,
119 without litter layer) were taken at about 50 cm distance from the stem of each tree. Soil samples
120 from each sub-plot were subsequently mixed. The total number of soil samples was 18, with six
121 species and three subplots per species. Field moist soil samples were brought to the laboratory in a
122 box filled with ice. All samples were sieved (1mm) and gravimetric soil moisture was determined
123 for each sample. Some chemical characteristics of the soil samples are presented in Table 1.

124

125 *2.2. Incubation experiment*

126 Soil samples (3 g) were placed into 120-mL flasks sealed with rubber stoppers. Distilled water was
127 added to adjust soil moisture to 60% of water holding capacity (WHC) and incubation was carried
128 out during one month at three levels of CH_4 addition to the headspace (30, 200 and 1000 ppm).

129 Every three days the flasks were opened for 30 min to avoid anaerobic conditions developing, and
130 were then again sealed and supplemented with CH_4 to give the concentrations given above. The
131 headspace was sampled every day for CH_4 concentration using a GC fitted with FID (SRI
132 Instruments, Inc., USA), and the consumption rate was estimated for every three days using linear
133 regression.

134

135 *2.3. PLFA extraction and ^{13}C determination*

136 Lipids were extracted from 3 g of soil by a modified Bligh and Dyer method (Bligh and Dyer 1959)
137 and fractionated on silica columns (CUSIL15Z; ICT, Bad Homburg, Germany). PLFAs were
138 subjected to mild alkaline methanolysis. Separation, identification, and quantification of fatty acid
139 methyl esters (FAMEs) were performed by gas chromatography mass spectrometry (GC-MS) on a
140 5% phenyl methyl silicone capillary column; length 50 m, inside diameter 0.2 mm [HP Ultra 2,
141 Hewlett-Packard] using the temperature program: 2 min at 150°C, increase of 4°C min^{-1} to 280°C
142 and finally 280°C for 11 min (Abraham et al., 1998). The positions of double bonds were
143 determined by analysis of dimethyl disulfide adducts. Carbon isotope ratios of the individual FAMEs
144 were determined by GC-IRMS on a Finnigan MAT 252 isotope ratio mass spectrometer (IRMS), in
145 triplicate. The IRMS was coupled via a combustion interface to a HP 5890 gas chromatograph. The
146 fatty acid methyl esters were separated with a Restek Rtx-2 column (60 m, 0.32 mm inner diameter,
147 0.25 µm film thickness). The column effluent was combusted on-line in an oxidation oven (copper,
148 nickel, platinum-catalyst, 980°C), passed through a reactor with elemental copper (600°C) for
149 reducing NO_x and removing surplus O_2. Combustion gas was dried by passage over a water-
150 permeable membrane (Nafion). To calculate isotope ratios ($\delta^{13}C$) for the PLFAs, $\delta^{13}C$ values of the
151 FAMEs were corrected by mass balance for the carbon atom of the methyl group that was added
152 during methanolysis (Abrajano et al., 1994).

153

154 *2.4. Estimation of relative incorporation of ^{13}C*

155 For each PLFA, the incorporation of ^{13}C (I, expressed as picograms of ^{13}C per gram of total PLFAs)
156 was calculated as follows: $I = (F_l - F_u) \times (A_x)$, where A_x was the peak area of $PLFA_x$ divided by the
157 sum of the peak areas of all of the PLFAs. F was the fraction of ^{13}C in $PLFA_x$ of samples incubated
158 with ^{13}C (F_l) or samples incubated with ^{12}C (F_u) and was calculated as follows: $F = {}^{13}C/({}^{13}C + {}^{12}C) =$
159 $R/(R + 1)$. The carbon isotope ratio (R) was derived from the measured $\delta^{13}C$ values as follows: $R =$
160 $(\delta^{13}C/1{,}000 + 1) \times R_{VPDB}$, with $R_{VPDB} = 0.\ 0112372 \pm 0.0000090$.

161

162 *2.5. Statistical analysis*

163 The effect of tree species on CH_4 oxidation rates was studied using two-way ANOVA with tree
164 species and CH_4 concentration as independent factors. Spearman coefficients were computed to
165 study relationship between ^{13}C incorporated into all PLFAs and the rates of CH_4 oxidation. The
166 significance of tree species effects on the ratios of $^{13}C18{:}1\omega7$-to-$^{13}C16{:}0$ were tested for three levels
167 of CH_4 concentration with one-way ANOVA. All statistical procedures were performed using the
168 STATISTICA package.

169

170 **3. Results**

171 Methane oxidation rates were significantly affected by tree species, initial CH_4 concentration,
172 and the interaction between these two factors. Tree species affected the CH_4 oxidation rates at 30
173 ppm ($P<0.001$) and 200 ppm ($P<0.050$), but not at 1000 ppm ($P>0.050$) (Fig. 1). The CH_4 oxidation
174 rates at 30 and 200 ppm were constant during the incubation, but at 1000 ppm the rates increased
175 over time (data not shown). The growth of methanotrophs initiated by the highest CH_4 concentration
176 masked the effect of tree species at 1000 ppm treatment and made the main effect of tree species
177 insignificant ($P>0.050$). At 30 and 200 ppm, tree species affected the rates of CH_4 uptake similarly:
178 the rates were highest in soils beneath larch and Arolla pine, followed by deciduous aspen and birch
179 and lowest under spruce and Scots pine. Overall, the activity of CH_4 oxidation at low concentrations

180 varied between different tree species over a three-fold range. As expected, higher initial
181 concentration of CH_4 significantly increased the rate of CH_4 oxidation ($P<0.001$) (Fig. 1). Compared
182 to the rate of CH_4 oxidation at 30 ppm the rates increased over time by factor of 2 at 200 ppm and
183 by a factor of 22 at 1000 ppm.

184 The amount of ^{13}C incorporated into PLFA linearly increased with the amount of CH_4 oxidized
185 at all CH_4 concentrations (Fig. 2). The correlation enables estimation of the carbon use efficiency
186 (fraction of C incorporated into PLFAs of CH_4 oxidized) at the different initial CH_4 concentrations.
187 The values of carbon use efficiency were similar for 30 and 200 ppm (153 and 138 $\mu g^{13}C$
188 incorporated per g PLFAs/ nmol CH_4 oxidized g^{-1} soil day^{-1}, respectively) and were approximately
189 100-150 times higher for 1000 ppm (Fig. 2). Overall, the PLFAs were enriched ca. 2000 times more
190 at 1000 ppm than at lower concentrations of CH_4 owing to higher CH_4 oxidation rates (ca. by 20
191 times) and to higher carbon use efficiency. Different carbon use efficiency at 30/200 ppm and 1000
192 ppm is due to the different ^{13}C assimilation when the cells are not growing or growing, respectively.

193 At 30 and 200 ppm, most of ^{13}C (98%) was incorporated into only two PLFAs, 18:1ω7 and 16:0
194 (Fig. 3), the amounts of ^{13}C incorporated into the two PLFAs were strongly correlated (Fig. 4).
195 About nine times more ^{13}C was incorporated in 18:1ω7 than in 16:0 and the ratios of ^{13}C18:1ω7 to
196 ^{13}C16:0 were stable and independent on tree species ($P>0.050$). At 1000 ppm, on the other hand,
197 more types of PLFA became labeled (data not shown).

198 At 30 ppm, the CH_4 oxidation rates significantly correlated with the net N mineralization and
199 net nitrification rates (Fig. 5) and both relationships were positive; the maximum CH_4 oxidation
200 rates were observed under Arolla pine and larch, where also the highest net N mineralization and
201 nitrification rates were measured.

202

203 4. Discussion

204

205 *4.1. Tree species affect the activity of methanotrophs*

206 The aim of the current work was to determine the extent to which the structure of the microbial
207 community of high-affinity methanotrophs was responsible for tree species effects on atmospheric

208 CH_4 consumption. Tree species is known to strongly affect CH_4 consumption by soil (Borken et al.,
209 2003; Menyailo and Hungate, 2003; Reay et al., 2004; Borken and Beese, 2006), but whether this is
210 due to tree species effects on microbial CH_4 oxidation or soil gas diffusivity is not known. The
211 observed tree species effect has often been attributed to a different gas diffusivity of the litter layers
212 formed by deciduous and coniferous stands (Borken et al., 2003; Borken and Beese 2006). In our
213 laboratory incubations, gas diffusivity was kept as a constant and we found, that under these
214 conditions, the CH_4 consumption at low concentrations differed between tree species by a factor of
215 three, a much larger difference than observed in the field. For example, field measurements of CH_4
216 consumption on our experimental site revealed that only two species deviated significantly from the
217 other four: spruce had 20-25% lower and Arolla pine 10-20% higher consumption rates than all
218 other species (Menyailo et al. 2008). The present incubation study indicated that the difference in
219 microbial activities between different tree species can be as large as the difference in the field CH_4
220 uptake rates.

221 If two soil samples, under the same environmental conditions and with similar substrate
222 availability, show different rates of the process they might hypothetically be different in either
223 species composition (Zak et al., 2003), diversity (Groffman and Bohlen, 1999; Nannipieri et al.,
224 2003), or microbial biomass (Bengtsson, 1998). We expected, therefore, that at least one of the
225 parameters should be changed by tree species in our soils.

226 At low concentration of CH_4 (30 and 200 ppm) the labeling pattern of the PLFAs under all tree
227 species was very similar: a larger amount of ^{13}C was incorporated into 18:1ω7 and a smaller amount
228 into 16:0. The labeled PLFAs indicate that methanotrophs belonging to the Type II group were
229 active in our soils (Knief et al., 2003; Crossman et al., 2005) and the similar PLFAs labeled under
230 different tree species suggest that the composition of high affinity methanotrophs was not affected
231 by tree species. Changes in microbial composition that are detectable by PLFA analysis can thus not
232 explain the different CH_4 oxidation rates. However, we cannot rule out that the community
233 composition was different at a more refined level, which could only have been detected by SIP
234 analysis of microbial RNA or DNA.

235

236 *4.2. CH_4 oxidation correlates with nitrogen mineralization and nitrification*

237 Our previous studies at the Siberian afforestation site revealed that net nitrification and net N
238 mineralization in soil were most strongly affected by the different tree species (Menyailo et al.,
239 2002b; Menyailo, 2009). All these activities were highest under Arolla pine and larch, intermediate
240 under deciduous aspen and birch, and lowest beneath spruce and Scots pine (Menyailo et al., 2002b;
241 Menyailo, 2009). A plot of CH_4 oxidation rates at 30 ppm versus net N mineralization and net
242 nitrification rates (Fig. 5) revealed a significant positive correlation suggesting that N turnover
243 processes may be linked to the CH_4 oxidation.

244 While positive effects of N have been reported for CH_4 oxidation in a rice field soil (Bodelier et
245 al., 2000), in most cases the oxidation of atmospheric CH_4 in forest soils was inhibited by N addition
246 (Bodelier and Laanbroek, 2004; Mohanty et al., 2006). In our case the correlation of CH_4 oxidation
247 with inorganic N production was positive. It might be that an increase of inorganic N concentration
248 in soil due to N application or to inorganic N accumulation through internal soil processes, e.g.
249 nitrification and N mineralization, have different effects on the oxidation of atmospheric CH_4. We
250 acknowledge that the observed correlation between CH_4 oxidation rates and N production processes
251 could be simply due to co-variation and the effect might be an indirect one. For example, the rates of
252 net nitrification and net N mineralization were correlated to soil pH (Table 1, Menyailo et al.,
253 2002b).

254 In the recent study of Maurer et al. (2008) it was shown that high production of monoterpenes,
255 in particular α- and β-pinene and limonene, by roots of Norway spruce could lead to a reduction in
256 CH_4 oxidation activity. In the present work, we excluded roots and litter from our soil incubations.
257 However, we cannot exclude that monoterpenes were left in the soil from previous production in the
258 field and were thus still affecting CH_4 oxidation activity.

259 *4.3. Who was oxidizing atmospheric methane?*

260 The taxa of the methanotrophs, whose PLFAs became labeled at low CH_4 concentrations cannot
261 be determined to a species or genera level, because PLFA-SIP does not have as fine resolution as
262 SIP-DNA/RNA. Nevertheless, labeling of 18:1ω7 and the 16:0 PLFAs is characteristic for type II
263 methanotrophic bacteria, in particular to *Methylocapsa acidophila* and *Methylocella palustris*

264 (Dedysh et al., 2002; Crossman et al., 2005; Maxfield et al., 2006). *Methylocystis or Methylosinus*
265 species can probably be excluded from the dominant atmospheric CH_4 oxidizers from our soils,
266 because they have a high concentration of 18:1*ω*8c fatty acid instead of 18:1*ω*7c acid, which was
267 labeled in our soils and which dominates the profiles of the *Methylocella* and *Methylocapsa* bacteria
268 (Crossman et al., 2005).

269 The PLFA labeling patterns in our Siberian soils were similar to those observed in other
270 European soils (Roslev and Iversen, 1999; Bull et al., 2000; Crossman et al., 2005; Maxfield et al.,
271 2006; Knief et al., 2003, 2006), where labeling of 18:1ω7 and 16:0 PLFAs at near atmospheric CH_4
272 concentrations was associated with the presence of USCα (Knief et al., 2003, 2006). Metagenomic
273 data has recently shown that USCα is also closely related to *Methylocapsa acidophila* (Ricke et al.,
274 2005), supporting our assumption that labeling of 18:1ω7 and the 16:0 PLFAs is associated with the
275 presence of methanotrophs belonging to the USCα. Methanotrophs of USCγ were less likely to be
276 active in our soils since they are closely related to Type I methanotrophs, having PLFAs which were
277 not labeled in our soils. Also, organisms described as USCγs are often detected in soils with pH>6.0
278 (McDonald et al., 2008) rather than in more acidic soils, such as ours, where the pH_{H2O} varied from
279 5.5 to 6.0 (Menyailo et al., 2002b). Overall, representatives of USCα might be a dominant CH_4
280 consumer in the Siberian soils studied, but final proof would require molecular studies on the level
281 of *pmoA* genes, by which to date uncultured USCα is defined.
282

283 *4.4. Hypothesis on low diversity of USCα*

284 One major result of our work is the suggestion that tree species strongly affected activity of
285 atmospheric CH_4 consumption without apparently altering biomass and, most importantly, the
286 composition of high-affinity methanotroph community. This is very surprising since tree species
287 have been shown to strongly alter the composition of bacteria both in canopies (Lambais et al.,
288 2006) and in soil (Lejon et al., 2005). We submit that the lack of tree species effect is due to low
289 diversity of methanotrophs oxidizing atmospheric CH_4 in our soils. Since we have argued above that
290 members of the USCα were most likely responsible for atmospheric CH_4 oxidation in our soils, we

291 present below three lines of evidence that support the suggested by Kolb et al. (2005) hypothesis on
292 low diversity of members of the USCα.

293 First, in contrast to other ^{13}C labeling studies (Bull et al., 2000; Crossman et al., 2005; Knief et
294 al., 2003, 2006; Maxfield et al., 2006) our present work covers more soil samples (n=18) with a wide
295 range of CH_4 oxidation activities. This enabled detection of the strong positive correlation of ^{13}C
296 incorporated into 18:1ω7 versus 16:0 (Fig. 3), a pattern which was largely overlooked in other
297 studies. Independent of the tree species, nine times more ^{13}C was incorporated into 18:1ω7 than into
298 16:0. It is important to note that pure cultures of known species of methanotrophs vary widely in
299 PLFAs composition and have a different ^{13}C incorporation patterns (Bowman et al., 1993; Dedysh et
300 al., 2002). Therefore, it is very unlikely that there are many uncultivated and distant taxa, which
301 have identical PLFAs composition and strictly identical patterns of C incorporation. Most likely is
302 the dominance of only one taxa of high affinity methanotrophs, which oxidize atmospheric CH_4 in
303 our soils and in the range of upland forest soils in Europe (Bull et al., 2000; Crossman et al., 2005;
304 Knief et al., 2003, 2006; Maxfield et al., 2006).

305 Second, recent metagenomic analysis also suggests that the USCα is genetically a very narrow
306 group of organisms. Ricke et al., (2005) using two different primer sets targeting either *pmoA* of
307 USCα or all *pmoA* and *amoA* sequence types selected the two inserts containing *pmoA* sequences
308 out of 250,000 fosmid clones, taken from an upland forest soil. The shotgun-based sequencing
309 revealed that both inserts belonged to the same or to the two highly related USCα genotypes, since
310 the sequences obtained for the two inserts exhibited 100% identity over the overlapping region of 30
311 kb (Ricke et al., 2005). This provides evidence that the diversity of USCα in the forest soil studied
312 was low.

313 Third, in microbial ecology, the popular hypothesis of functional redundancy holds that in soil
314 there are so many species with similar function that loss of one species will not alter the way that the
315 system operates (Soil microbiology.., 2007). This hypothesis can be reformulated; if only few or
316 only one species can carry out the process the loss of that species will strongly damage ecosystem
317 function. The consumption of atmospheric CH_4 is one of the most unstable soil processes. For
318 example, several authors have reported loss of CH_4 oxidation after drying of soil (e.g. Dobbie and

319 Smith, 2006) or after disturbance by ploughing (Mosier et al., 1997) suggesting that the
320 methanotrophs involved in atmospheric CH_4 uptake have a very weak resistance to stress. Resilience
321 is also considered to be low with, for instance, Mosier et al. (1997) observing that cultivated soils
322 that had been converted back into grassland attained about one-third of the CH_4 uptake rate of
323 natural grassland after a period of 50 years. Both low resistance and resilience of soil CH_4 uptake
324 support the hypothesis of a low diversity for atmospheric CH_4 oxidizers.

325

326 *4.5. Conclusions*

327 We have shown that Siberian tree species strongly affect the rate of atmospheric CH_4 oxidation;
328 however, the effect appears not to be due to major changes in composition of the active
329 methanotrophic community, as evidenced by ^{13}C PLFA labeling patterns, which were surprisingly
330 uniform across the Siberian site. We argue that the apparently low diversity of high affinity
331 methanotrophs, presumably belonging to the USCα, explains the absence of tree species effect on
332 the composition CH_4 oxidizers in our soils.

333

334 Acknowledgements

335 We thank Esther Surges for the isotope ratio measurements, Svetlana Dedysh and Peter Frenzel for
336 discussion of the data. The funding was provided by the Alexander von Humboldt Foundation,
337 Marie Curie Fellowship and by the Russian President Award for best professors awarded to OVM.

338

339 References

340 Abraham, W.-R., Hesse, C., Pelz, O., 1998. Ratios of carbon isotopes in microbial lipids as an
341 indicator of substrate usage. Applied and Environmental Microbiology 64, 4202-4209.

342 Abrajano, T.A. jr., Murphy, D.E., Fang, J., Comet, P., Brooks, J.M., 1994. $^{13}C/^{12}C$ ratios in
343 individual fatty acids of marine mytilids with and without bacterial symbionts. Organic
344 Geochemistry 12, 611-617.

345 Angers, D.A., Caron, J., 1998. Plant-induced changes in soil structure: processes and feedbacks.
346 Biogeochemistry 42, 55–72.
347 Baani, M., Liesack, W., 2008. Two isozymes of particulate methane monooxygenase with different
348 methane oxidation kinetics are found in *Methylocystis* sp. strain SC2. Proceeding of the
349 National Academy of Sciences USA 105, 10203-10208.
350 Bengtsson, J., 1998. Which species? What kind of diversity? Which ecosystem function? Some
351 problems in studies of relations between biodiversity and ecosystem function. Applied Soil
352 Ecology 10, 191-199.
353 Bligh, E.G., Dyer, W.J., 1959. A rapid method of total lipid extraction and purification. Canadian
354 Journal of Biochemistry and Physiology 37, 911-917.
355 Bodelier, P.L.E., Roslev, P., Henckel, T., Frenzel, P., 2000. Stimulation by ammonium-based
356 fertilizers of methane oxidation in soil around rice roots. Nature 403, 421-424.
357 Bodelier, P.L.E., Laanbroek, H.J., 2004. Nitrogen as a regulatory factor of methane oxidation in
358 soils and sediments. FEMS Microbiology Ecology 47, 265-277.
359 Bodelier, P.L.E., Gillisen, M.-J.B., Hordijk, K., Damsté, J.S.S., Rijpstra, W.I.C., Geenevasen, J.A.J.,
360 Dunfield, P.F., 2009. A reanalysis of phospholipid fatty acids as ecological biomarkers for
361 methanotrophic bacteria.The ISME journal 3, 606-617.
362 Borken, W., Beese, F., 2006. Methane and nitrous oxide fluxes of soils in pure and mixed stands of
363 European beech and Norway spruce. European Journal of Soil Science 57, 617-625.
364 Borken, W., Xu, Y. J., Beese, F., 2003. Conversion of hardwood forests to spruce and pine
365 plantations strongly reduced soil methane sink in Germany, Global Change Biology 9, 956-
366 966.
367 Bowman, J.P., Sly, L.I., Nichols, P.D., Hayward, A.C., 1993. Revised taxonomy of the
368 methanotrophs: description of *Methylobacter* gen. nov., emendation of *Methylococcus*,
369 validation of *Methylosinus* and *Methylocystis* species, and a proposal that the family

370 *Methylococcaceae* includes only the group I methanotrophs, International Journal of
371 Systematic Bacteriology 43, 735–753.
372 Bremer, C., Braker, G., Matthies, D., Reuter, A., Engels, C., Conrad, R., 2007. Impact of plant
373 functional group, plant species, and sampling time on the composition of nirK-type denitrifier
374 communities in soil. Applied and Environmental Microbiology 73, 6876-6884.
375 Bull, I.D., Parekh, N.R., Hall, G.H., Ineson, P., Evershed, R.P., 2000. Detection and classification of
376 atmospheric methane oxidizing bacteria in soil. Nature, 405, 175-178.
377 Carney, K.M., Matson, P.A., 2006. The influence of tropical plant diversity and composition on soil
378 microbial communities. Microbial Ecology 52, 226-238.
379 Crossman, Z. M., Ineson, P., Evershed, R. P., 2005. The use of ^{13}C labelling of bacterial lipids in
380 characterization of ambient methane-oxidizing bacteria in soil. Organic Geochemistry 36, 769-
381 778.
382 Dedysh, S.N., Khmelenina, V.N., Suzina, N.E., Trotsenko, Y.A., Semrau, J.D., Liesack, W., Tiedje,
383 J.M., 2002. *Methylocapsa acidiphila* gen. nov., sp. nov., a novel methane-oxidizing and
384 dinitrogen-fixing acidophilic bacterium from *Sphagnum* bog. International Journal of
385 Systematic Bacteriology 52, 251–261.
386 Dobbie, K.E., Smith, K. A., 2006. Comparisons of CH_4 oxidation rates in woodland, arable and set
387 aside soils. Soil Biology and Biochemistry 28, 1357-1365.
388 Grayston, S.J., Prescott, C.E., 2005. Microbial communities in forest floors under four tree species
389 in coastal British Columbia. Soil Biology and Biochemistry 37, 1157-1167.
390 Hackl, E., Zechmeister-Boltenstern, S., Bodrossy, L., Sessitsch, A., 2004. Comparison of diversities
391 and compositions of bacterial populations inhabiting natural forest soils. Applied and
392 Environmental Microbiology 70, 5057–5065.
393 Hanson, R.S., Hanson, T. E., 1996. Methanotrophic bacteria. Microbiology Review 60, 439–471.

394 Hooper, D.U., Bignell, D.E., Brown, V.K., Brussaard, L., Dangerfield, J.M., Wall, D.H., Wardle,
395 D.A., Coleman, D.C., Giller, K.E., Lavelle, P., van der Putten, W.H., de Ruiter, P.C., Rusek,
396 J., Silver, W., Tiedje, J.M., Wolters, V., 2000. Interactions between above- and belowground
397 biodiversity in terrestrial ecosystems: Patterns, mechanisms, and feedbacks. BioScience 50,
398 1049-1061.

399 IPCC, 2007: Climate Change 2007: The Physical Science Basis. Contribution of Working Group I
400 to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change
401 [Solomon, S., D. Qin, M. Manning, Z. Chen, M. Marquis, K.B. Averyt, M. Tignor and H.L.
402 Miller (eds.)]. Cambridge University Press, Cambridge, United Kingdom and New York, NY,
403 USA.

404 Knief, C., Dunfield, P.F., 2005. Response and adaptation of methanotrophic bacteria to low methane
405 concentrations. Environmental Microbiology 7, 1307-1317.

406 Knief, C., Kolb, S., Bodelier, P.L., Lipski, A., Dunfield, P.F., 2006. The active methanotrophic
407 community in hydromorphic soils changes in response to changing methane concentration.
408 Environmental Microbiology 8, 321-333.

409 Knief, C., Lipski, A., Dunfield, P.F., 2003. Diversity and activity of methanotrophic bacteria in
410 different upland soils. Applied and Environmental Microbiology 69, 6703–6714.

411 Kolb, S., Knief, C. & Dunfield, P.F., Conrad, R., 2005. Abundance and activity of uncultured
412 methanotrophic bacteria involved in the consumption of atmospheric methane in two forest
413 soils. Environmental Microbiology 7, 1150-1161.

414 Lambais, M.R., Crowley, D.E., Cury, J.C., Bull, R.C., Rodrigues, R.R., 2006. Bacterial diversity in
415 tree canopies of the Atlantic forest. Science 312, 1917.

416 Lau, E., Ahmad, A., Steudler, P.A., Cavanaugh, C.M., 2007. Molecular charachterization of
417 methanotrophic communities in forest soils that consume atmospheric methane. *FEMS*
418 *Miocrob. Ecol.*, 60, 490-500.

419 Lejon, D.P.H., Chaussod, R., Ranger, J., Ranjard L., 2005. Microbial community structure and
420 density under different tree species in an acid forest soil (Morvan, France). Microbial Ecology
421 50, 614–625.
422 Maurer, D, Kolb, S., Haumaier, L., Borken, W. 2008. Inhibition of atmospheric methane oxidation
423 by monoterpenes in Norway spruce and European beech soils, Soil Biology and Biochemistry
424 40, 3014-3020.
425 Maxfield, P.J., Hornibrook, E.R.C., Evershed, R.P., 2006. Estimating high-affinity methanotrophic
426 bacterial biomass, growth, and turnover in soil by phospholipid fatty acid ^{13}C labeling.
427 Applied and Environmental Microbiology 72, 3901- 3907.
428 McDonald, I.R., Bodrossy, L., Chen, Y., Murrell J.C., 2008. Molecular ecology techniques for the
429 study of aerobic methanotrophs. Applied and Environmental Microbiology 74, 1305-1315.
430 Menyailo, O.V., 2009. The effect of Siberian tree species on the mineralization rate of soil organic
431 matter. Eurasian Soil Science 42, 1156-1162.
432 Menyailo, O.V., Hungate, B.A., Abraham, W.-R., Conrad, R., 2008. Changing land use reduces soil
433 CH_4 uptake by altering biomass and activity but not composition of high-affinity
434 methanotrophs. Global Change Biology 14, 2405-2419.
435 Menyailo, O.V., Hungate, B.A., 2003. Interactive effects of tree species and soil moisture on
436 methane consumption. Soil Biology and Biochemistry 35, 625-628.
437 Menyailo, O.V., 2007. The influence of tree species on the biomass of denitrifying bacteria in gray
438 forest soils. Eurasian Soil Science 3, 302 - 307.
439 Menyailo, O.V., Hungate, B.A., Zech, W., 2002a. Tree species mediated soil chemical changes in a
440 Siberian artificial afforestation experiment, Plant and Soil 242, 171-182.
441 Menyailo, O.V., Hungate, B.A., Zech, W., 2002b. The effect of single tree species on soil microbial
442 activities related to C and N cycling in the Siberian artificial afforestation experiment. Plant
443 and Soil 242, 183-196.

444 Mohanty, S.R., Bodelier, P.L.E., Floris, V., Conrad R., 2006. Differential effects of nitrogenous
445 fertilizers on methane-consuming microbes in rice field and forest soil. Applied and
446 Environmental Microbiology 72, 1346-1354.
447 Mosier, A.R., Parton, W.J., Valentine, D.W., Ojima, D.S., Schimel, D.S., Heinemeyer, O., 1997.
448 CH_4 and N_2O fluxes in Colorado shortgrass steppe. 2. Long-term impact of land use change.
449 Global Biogeochemical Cycles 11, 29-42.
450 Nannipieri, P., Ascher, J., Ceccherini, M.T., Landi, L., Pietramellara, G., Renella, G., 2003.
451 Microbial diversity and soil functions. European Journal of Soil Science 54, 655-670.
452 Nugroho, R.A., Röling, W.F.M., Laverman, A.M., Verhoef, H.A., 2006. Net nitrification rate and
453 presence of *Nitrosospira* cluster 2 in acid coniferous forest soils appear to be tree species
454 specific. Soil Biology and Biochemistry 38, 1166-1171.
455 Priha, O., Smolander A., 1997. Microbial biomass and activity in soil and litter under *Pinus*
456 *sylvestris*, *Picea abies* and at originally similar field afforestation sites. Biology and Fertility
457 of Soils 24, 45-51.
458 Ricke, P., Kube, M., Nakagawa, S., Erkel, C., Reinhard, R., Liesack, W., 2005. First genome data
459 from uncultured upland soil cluster alpha methanotrophs provide further evidence for a close
460 phylogenetic relationship to *Methylocapsa acidophyla* B2 and for high-affinity methanotrophy
461 involving particulate methane monooxygenase. Applied and Environmental Microbiology 71,
462 7472- 7482.
463 Roslev, P., Iversen, N., 1999. Radioactive fingerprinting of microorganisms that oxidize
464 atmospheric methane in different soils. Applied and Environmental Microbiology 65, 4064-
465 4070.
466 Smith, K.A., Ball, T., Conen, F., Dobbie, K.E., Massheder, J., Rey, A., 2003. Exchange of
467 greenhouse gases between soil and atmosphere: interactions of soil physical factors and
468 biological processes. European Journal of Soil Science 54, 779-791.

469 Soil Microbiology, Ecology and Biochemistry, 2007. Paul, E.A., (ed.), 3rd ed. Academic Press,
470 London, 532 p.
471 Zak, D.R., Holmes, W.E., White, D.C., Peacock, A.D., Tilman, D., 2003. Plant diversity, soil
472 microbial communities, and ecosystem function: Are there any links? Ecology 84, 2042-2050.
473
474
475
476
477
478
479
480
481
482
483
484
485
486
487
488
489
490
491
492
493

494 **Legends to Figures.**

495 **Figure 1.** Average rates of CH_4 oxidation by soils sampled from beneath different tree species and
496 incubated at three initial CH_4 concentrations. Mean values and standard errors are presented for
497 three replicates. The significance of tree species effect is given for each CH_4 concentrations, the
498 main effects and their interactions are given in the text.

499

500 **Figure 2.** Plot of ^{13}C incorporated into PLFAs versus CH_4 oxidation rates for three concentrations of
501 CH_4: 30 ppm – dark circles, 200 ppm – white squares and 1000 ppm - dark squares. Since the
502 amount of CH_4 oxidized is estimated per g of soil and ^{13}C icorporation is presented per g of total
503 PLFAs, strong correlation suggests that the amount of PLFAs is not different among soils.

504

505 **Figure 3.** ^{13}C incorporation into the two individual PLFAs (16:0 and 18:1ω7c) in soil samples
506 incubated under 30 or 200 ppm of CH_4. Mean values and standard errors are presented for three
507 replicates.

508

509 **Figure 4.** Plot of ^{13}C incorporated into 16:0 versus 18:1ω7 PLFAs in soil sampled from beneath
510 different trees and incubated at 30 ppm CH_4 (open circles) and 200 ppm CH_4 (dark circles).

511

512 **Figure 5.** Plot of CH_4 oxidation rates determined at 30 ppm of CH_4 versus net nitrification and net N
513 mineralization rates in soils sampled from beneath different trees. The values for net N
514 mineralization and net nitrification are taken from Menyailo et al., 2002b. Net N mineralization was
515 determined as a difference in [NH_4^+ + NO_3^-] before and after 30 days incubation of soil samples. Net
516 nitrification was detemined as a difference in [NO_3^-] before and after incubation. Mean values and
517 standard errors are presented for three replicates.

518 Table 1. Some chemical properties of studied soils beneath the different tree species in Siberian afforestation experiment. Values are means
519 of three plots; standard errors are given in parentheses; values in one row followed by the same letter are not significantly different
520 from each other ($P < 0.05$).

Chemical properties	Tree species effect, *P*	Spruce	Scots pine	Arolla pine	Larch	Aspen	Birch
pH	<0.0001	5.55 (0.10)[c]	5.88 (0.02)[b]	6.02 (0.09)[a]	6.06 (0.02)[a]	5.82 (0.03)[b]	5.80 (0.04)[b]
NH_4^+, mg kg^{-1}	<0.0001	4.62 (1.62)[b]	4.96 (0.34)[b]	27.68 (5.97)[a]	7.98 (1.23)[b]	6.20 (0.33)[b]	6.49 (2.30)[b]
NO_3^-, mg kg^{-1}	0.277	1.14 (1.19)	0.63 (1.09)	4.47 (4.00)	6.81 (2.90)	3.25 (4.67)	4.05 (3.98)
N, %	0.395	0.18 (0.01)	0.18 (0.01)	0.27 (0.16)	0.31 (0.15)	0.22 (0.01)	0.18 (0.01)
C, %	0.458	3.24 (0.31)	2.86 (0.04)	4.14 (2.61)	4.89 (2.46)	3.24 (0.12)	2.65 (0.13)
C/N	0.002	17.58 (1.14)[a]	16.34 (0.96)[ab]	15.01 (0.85)[c]	15.73 (0.45)[bc]	14.68 (0.27)[c]	14.49 (0.31)[c]
DON, mg kg^{-1}	0.421	17.50 (3.75)	15.53 (1.36)	17.05 (1.99)	17.85 (0.09)	17.68 (1.50)	15.17 (0.54)
DOC, mg kg^{-1}	0.834	277.67 (74.80)	244.50 (45.67)	276.83 (42.05)	281.00 (15.40)	291.00 (31.00)	257.83 (46.52)

521