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SHORT COMMUNICATION

Response of methanotrophic communities to afforestation and reforestation in New Zealand

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Methanotrophs use methane (CH_4) as a carbon source. They are particularly active in temperate forest soils. However, the rate of change of CH_4 oxidation in soil with afforestation or reforestation is poorly understood. Here, soil CH_4 oxidation was examined in New Zealand volcanic soils under regenerating native forests following burning, and in a mature native forest. Results were compared with data for pasture to pine land-use change at nearby sites. We show that following soil disturbance, as little as 47 years may be needed for development of a stable methanotrophic community similar to that in the undisturbed native forest soil. Corresponding soil CH_4-oxidation rates in the regenerating forest soil have the potential to reach those of the mature forest, but climo-edaphic fators appear limiting. The observed changes in CH_4-oxidation rate were directly linked to a prior shift in methanotrophic communities, which suggests microbial control of the terrestrial CH_4 flux and identifies the need to account for this response to afforestation and reforestation in global prediction of CH_4 emission.

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Globally, methane (CH_4) constitutes the second most abundant greenhouse gas after carbon dioxide (CO_2) (IPCC, 2007). Despite a short atmospheric lifetime of approximately 8–12 years, CH_4 is at least 25 times more potent than CO_2 (Shindell *et al.*, 2009). The only terrestrial sink for atmospheric CH_4 occurs through the activity of high-affinity CH_4-oxidising bacteria (methanotrophs) (Hanson and Hanson, 1996; Trotsenko and Murrell, 2008) with 30–50% of this sink occurring in temperate forest soils (Ojima *et al.*, 1993). New Zealand (NZ) forest soils, especially from pristine temperate forests, have been shown to be strong atmospheric CH_4 sinks compared with most Northern Hemisphere forest soils (Price *et al.*, 2003). This has been attributed to NZ's isolation, the low rate of atmospheric nitrogen deposition and limited anthropogenic soil disturbance. Changes in land use and management are known to alter the composition of the methanotrophic community and, therefore, influence CH_4 oxidation (Ojima *et al.*, 1993; MacDonald *et al.*, 1997). Clear-felling and deforestation change the soil from a net sink for CH_4 to a net source (Keller *et al.*, 1990; Keller and Reiners, 1994; Zerva and Mencuccini, 2005), although Tate *et al.* (2006) reported a rapid recovery of CH_4 oxidation after clear-felling of a pine forest with minimal soil disturbance. It has been calculated that the global soil-CH_4 sink has declined by 71% due to the conversion of natural soils for agricultural use and it is estimated that it could take >100 years for the soil-CH_4 sink strength of an afforested soil in Northern Europe to recover from disturbance by land-use change (Smith *et al.*, 2000). Only limited experimental evidence is available to improve prediction of changes in CH_4 emission due to afforestation and deforestation.

We studied a chronosequence of regenerating native forest (reforestation) and a mature native forest, both on similar volcanic ash soils at two sites in NZ. We combined these data with our earlier work at nearby sites on the effects on soil-CH_4 oxidation of recent afforestation of pastures with *Pinus radiata* (Singh *et al.*, 2007, 2009; Tate *et al.*, 2007). Our aims were (1) to determine the time required after reforestation for soils to achieve high CH_4-oxidation rates comparable to a mature native forest soil, and (2) to determine if the change in CH_4-oxidation rates related to a shift in methanotrophic communities at the ecosystem level.

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Table 1 Description of the different sites and land uses of the comparative analysis

Site[a]	*Land use*	*Name used in this study*[b]	*Description*	*Reference*
Turangi	Pasture	Pasture-5 ($n=4$)	Pasture adjacent to a 5-year-old pine forest	Singh *et al.* (2009)
		Pasture-10 ($n=4$)	Pasture adjacent to a 10-year-old pine forest	
	Pine forest (*Pinus radiata*)	Pine-5 ($n=4$)	5-year-old pine forest	
		Pine-10 ($n=4$)	10-year-old pine forest	
	Shrubland	Turangi-47 ($n=6$)	47-year-old shrubland	This study
		Turangi-67 ($n=6$)	67-year-old shrubland	
Puruki	Pasture	Pasture-7 ($n=3$)	Pasture adjacent to a 7-year-old (2nd rotation) pine forest	Singh *et al.* (2007); Tate *et al.* (2007)
	Pine forest (*Pinus radiata*)	Pine-7 ($n=3$)	7-year-old (2nd rotation) pine forest	
	Native forest	Puruki-Native ($n=9$)	Mature native forest	This study

[a]Soil physical data from Puruki-Native were compared to data from Pasture-7 and Pine-7. The soil chemical properties, CH_4 oxidation rates, phospholipid fatty acid composition, terminal-restriction fragment abundance and clone comparison, were also compared between the different land uses for both Turangi and Puruki sites.
[b]The number of samples used for each habitat is shown in brackets. The pastures correspond to the adjacent pasture of each pine stand. The age of the pasture and pine stand is also indicated by the number next to the land uses.

Details of field sites, methodology and statistical approaches are provided in the Supplementary Section. The two sites sampled for this study were a regenerating native forest (shrubland) after repeated burning, a common practice to develop pasture (Turangi, Tongariro National Park in central North Island, NZ (39°05′S, 175°45′E)) (Ross *et al.*, 2009), and a mature native forest (referred to as Puruki-Native) adjacent to pasture and exotic pine trees (*Pinus radiata*) (Puruki, Purukohukohu experimental basin in central North Island about 30 km south of Rotorua, NZ (38°26′S and 176°13′E)) (Tate *et al.*, 2006, 2007). At Turangi, two shrubland stands of 47 and 67 years (Manuka (*Leptospermum scoparium*) and Kanuka (*Kunzea ericoides*)) were selected and referred to here as Turangi-47 and Turangi-67, respectively. Results were compared with those from our previous studies (Singh *et al.*, 2007, 2009; Tate *et al.*, 2007) in nearby pasture and pine forests at Turangi, and at Puruki. A summary of the different land uses compared in this study is in Table 1.

We examined soil CH_4-oxidation rates and associated methanotrophic communities at these sites. CH_4 concentrations were measured by gas chromatography and fluxes calculated following Saggar *et al.* (2007) (see Supplementary Section for details). We also measured several soil chemical (pH, total C and N, organic N (NH_4^+-N and NO_3^--N)) and physical (moisture content, porosity, water-filled pore space and bulk density) properties. The Puruki-Native soil was better aerated (lower bulk density and water-filled pore space, and greater porosity) than adjacent soils (Tate *et al.*, 2007; Singh *et al.*, 2009) under pine trees and pasture ($P<0.001$). There was a trend towards better aeration in forest soils compared with pasture soils at both sites. Soil moisture content and concentrations of NO_3^--N and total N significantly decreased under pine. However, the soil C:N ratio increased with afforestation ($P<0.001$) but total C and NH_4^+-N concentrations and pH were unchanged (Supplementary Table 1).

Methane oxidation rates were significantly influenced by reforestation at Turangi, and by afforestation at the Puruki sites (Figure 1a). Our earlier work at a nearby Turangi site showed that although the soil CH_4 oxidation in young pine stands (5- and 10-years old) did not differ significantly from rates in the adjacent pastures, there was a clear shift to type-II-related methanotrophs in the pine soils (Singh *et al.*, 2009). In contrast to these pasture and young pine forest soils, CH_4-oxidation rates were significantly higher ($P<0.001$) at Turangi-47 and Turangi-67 shrubland, but were lower ($P<0.001$) than those in the Puruki-Native soil (Figure 1a). Nonetheless, our data suggest that CH_4-oxidation rates stabilised in Turangi shrubland after 47 years of reforestation as there was no apparent subsequent change over 20 years. Shrublands dominated by manuka and kanuka at previously disturbed sites are often seral communities, and in the absence of fire they are succeeded over 150–500 years by a permanent cover of tall forest (Ross *et al.*, 2009). However, it is likely that local climo-edaphic factors including very high annual rainfall (*ca.* 2500 mm) that periodically limits soil aeration, and very low N availability (Ross *et al.*, 2009), may be limiting further changes at the Turangi site. At the Puruki site (*ca.* 1500 mm of rainfall), CH_4-oxidation rates measured using large field chambers following clear-cutting of the nearby 24-year-old pine (Tate *et al.*, 2006) were comparable with rates in the Puruki-Native soil. This suggests that with minimal soil disturbance and high aeration status, soil CH_4-oxidation rates under second rotation pine (Pine-7, see Table 1) can reach those of a mature forest in as little as 31 years (first rotation to clear-cut, 24 years plus second rotation, 7 years). The high CH_4-oxidation rate from the Puruki-Native soil was also comparable to that of another pristine forest soil at Craigieburn in NZ South Island (Price *et al.*, 2003).

To examine whether changes in CH_4-oxidation rate were linked to a shift in the microbial community, we analysed soil methanotrophic communities

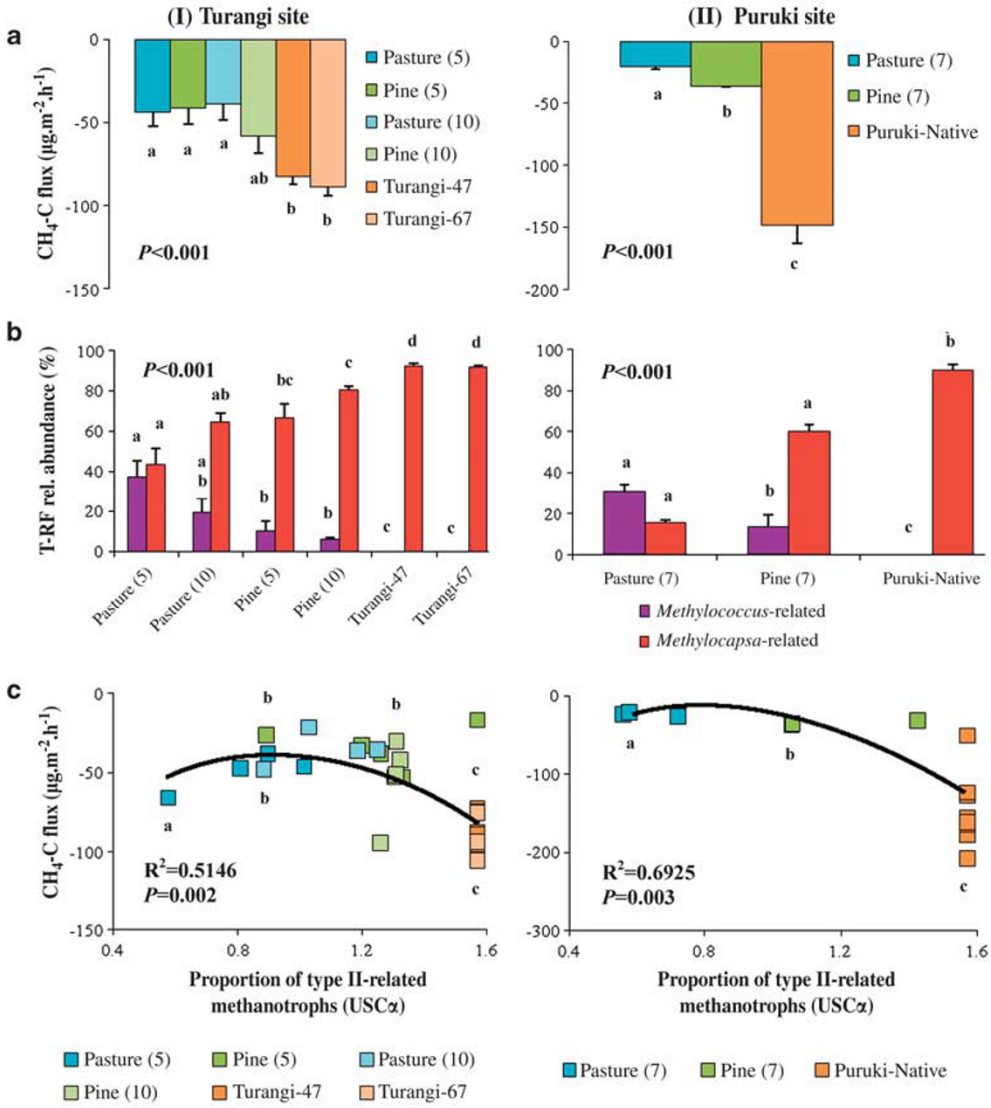

Figure 1 (**a**) Mean atmospheric CH_4 oxidation in soils from the different land uses at (I) Turangi; and (II) Puruki. (**b**) Means of the relative abundance of the dominant terminal-restriction fragments (T-RFs) in soils under the different land uses at Turangi and Puruki. Relative abundance of type-I-related methanotrophs was obtained from T-RF 245 (a relative of *Methylococcus* sp.) and type-II-related (or alphaproteobacterial) methanotrophs from T-RF 33 and T-RF 129 combined (distant relatives of USCα clone). Values for each land use did not add up to 100% because graphs only display the contribution of the T-RFs 33, 129 and 245. They cumulated >75% of the total T-RFs detected for each habitat. However, soils under pasture at Puruki (Pasture-7) contained a high proportion (~30%) of the T-RF 81, which was identified as being related to *Methylocystis* and *Methylosinus* spp. (Singh *et al.*, 2009). (**c**) Relationship between CH_4-oxidation rate and methanotrophic community structure at Turangi ($n=28$) and Puruki ($n=15$). The polynomial regression is based on the angular transformation (arcsine of the square root) of the proportion of the relative abundance of the dominant type-II-related T-RFs (T-RF 33 + T-RF 129) over the total relative abundance of the three dominant T-RFs (T-RFs 33, 129 and 245). Relative abundance was calculated as a percentage of the total number of T-RFs from each profile produced after digestion of the PCR products for *pmoA* genes with the enzyme *Hha*I. The error bars represent the s.e.m. CH_4-oxidation rates are those of the different land uses. Data for the pastures and pines at Turangi were taken from Singh *et al.* (2009), whereas CH_4 fluxes of pasture and pines at Puruki were from Tate *et al.* (2007). For each figure, land uses followed by different letters within a dataset (series) are statistically different according to the multiple pairwise comparison test ($\alpha=0.05$). Land-use colour legend: pastures are represented in blue, young forests (*Pinus radiata*) in green and old forests (shrublands and native) in orange. Colour shades show land uses of different age.

using terminal-restriction fragment length polymorphism of particulate methane monooxygenase (*pmoA*) genes (Singh *et al.*, 2007) using the primer pair A189–mb650 (Bourne *et al.*, 2001). Cloning and sequencing of *pmoA* genes from different samples of Turangi shrublands and Puruki-Native confirmed that terminal-restriction fragment (T-RF) 245 belonged to type-I methanotrophs and a close relative of *Methylococcus capsulatus,* while T-RF 33 and T-RF 129 were distant relatives of the uncultured clade USCα (a distant relative of cultured *Methylocapsa* sp., a type-II methanotroph)

(Supplementary Figure 1; sequence accession numbers FR715958 to FR715985). Based on the relative proportion of the three most dominant T-RFs, the relative dominance of type-II-related methanotrophs (T-RFs 33 and 129) increased with the age of the forest at the expense of type-I-related methanotrophs (T-RF 245) ($P<0.001$; Figure 1b; Supplementary Table 2). Regression analysis suggested that there was a significant relationship between the relative proportion of type-II-related methanotrophs and the rate of CH_4 oxidation at both sites (Figure 1c). These results provide strong evidence that the change in CH_4 oxidation was directly linked to a shift towards type-II methanotrophs, as previously suggested (Singh *et al.*, 2007; Dörr *et al.*, 2010). Our data also suggest that the soil methanotrophic community recovered first after afforestation/reforestation followed by the CH_4-oxidation rates, suggesting a microbial control of oxidation rate. Indeed, the relative abundance of the type-II-related methanotrophs in the 10-year-old pine forest was already close to that found in the older forests (Figure 1b), while CH_4-oxidation rate was at an intermediate stage (Figure 1a). Finally, we employed phospholipid fatty acid-stable isotope probing (PLFA-SIP) to identify the active methanotrophic populations. The most highly ^{13}C-enriched PLFA was C18:1ω7 (a signature for type II) at all sites (Supplementary Figure 2). Along with molecular data, this confirmed that type-II-related methanotrophs (USCα clones of the alphaproteobacterial class) were the most active in oxidising atmospheric CH_4 (Singh *et al.*, 2007; Dörr *et al.*, 2010). As a non-polar column was used, the PLFAs C18:1ω7c and C18:1ω8c could not be separated. C18:1ω8c is a signature PLFA of *Methylocystis* and *Methylosinus* spp. (Bodelier *et al.*, 2009), thus these may have incorporated ^{13}C into this PLFA. However, the T-RF 81, which was found to represent organisms related to *Methylocystis* and *Methylosinus* spp. (Singh *et al.*, 2007, 2009), was not detected in the terminal-restriction fragment length polymorphism profiles from any of the forest soils. Overall, our combined set of data from five sites (Singh *et al.*, 2007, 2009; Singh and Tate, 2007; this study) suggest that *Methylococcus capsulatus*-related (type I) and two relatives of USCα are the three most dominant genotypes in these NZ forest soils.

A limitation of our findings was that soils were sampled at different times. In a previous study (Price *et al.*, 2003), changes in soil moisture rather than temperature were responsible for most of the observed seasonal changes in soil CH_4 oxidation. In our volcanic soils, a combination of good aeration and moisture storage characteristics generally ensures these seasonal changes are quite small (Tate *et al.*, 2006). Consequently, to minimise any seasonality effects, we sampled all soils in this study only in summer (October to February).

This study provides the first experimental evidence that <47 years is potentially needed after afforestation/reforestation to establish a stable methanotrophic community equivalent to that of a mature native forest. However, local climo-edaphic factors that may be limiting vegetation succession to a mature forest may also limit methanotrophic activity. Our data suggest a niche-specific adaptation and microbial control of the observed changes in soil CH_4 oxidation. The mechanism associated would require the prior establishment of a type-II-related methanotrophic community before significant increase in CH_4-oxidation rates could occur. These significant findings need to be taken into consideration in future prediction of changes in CH_4 emissions resulting from afforestation and reforestation.

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