Distribution of Atmospheric Methane Oxidation and Methanotrophic Communities on Hawaiian Volcanic Deposits and Soils

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Hawaiian volcanic deposits offer ideal opportunities to assess methanotrophic bacterial colonization of new substrates, and to determine the relative significance of methanotrophy during ecosystem succession. Activity and molecular ecological surveys indicated that significant methanotrophic activity was restricted to vegetated ecosystems characterized by closed-canopy forests and significant soil accumulation. In these systems, atmospheric methane oxidation rates (0.7–1.8 mg CH4 m–2 d–1) were comparable to the lower end of values reported for continental soils. No trends in activity related to deposit age or type were evident at ambient or elevated methane levels. Analyses of clone libraries based on particulate methane monooxygenase and ammonia monooxygenase (pmoA/amoA) genes revealed largely novel sequences, with distinct assemblages for each of two sites. Remarkably, sequences from a 300-yr old forest soil were most closely related to sequences from Arctic soils. Collectively, the evidence indicates that methanotrophs colonize volcanic substrates slowly and likely depend on interactions with plant and other microbial communities.

Key words: methane, methanotroph, methane-oxidizing bacteria, soil, volcanic

Numerous studies have shown that grassland, forest, and other managed and unmanaged soils consume atmospheric methane5,7,17,22, collectively removing up to 50 Tg methane yr−1 globally17. Due to the importance of methane in tropospheric chemistry and radiative forcing, the distribution and kinetics of methane uptake along with responses to numerous environmental variables have been well documented1,2,4,9,19,20,24,25,28,30,35–38. The microbiology of atmospheric methane consumption has also been addressed using conventional cultivation and molecular methods5,8,12,13,15,22,23,28,30,34,42 but methanotrophs active in situ remain largely uncharacterized. Several lines of evidence suggest that novel forms occur commonly, and that community structure changes in response to changes in environmental variables4,24,29,39.

Responses of atmospheric methane uptake to anthropogenic disturbances indicate that methanotrophs are neither resistant nor resilient to change5,7,24,27,39. Several studies suggest that recovery from disturbances occurs over long periods, even decades9,40, but little is known about the ability of methanotrophs to colonize natural systems from which they are initially absent.

Recent volcanic deposits near Kilauea and Mauna Loa volcanoes (Hawaii) offer ideal environments for addressing questions about methanotroph colonization and succession. Results presented here show that atmospheric methane consumption is largely limited to sites with significant plant community development and soil formation. Molecular ecological analyses based on partial sequences of particulate methane monooxygenase genes (pmoA) amplified from genomic extracts of two soils also suggest that methanotrophic diversity is relatively limited, even in soils developing on lava flows >50,000 yr old. The distribution of methanotrophic activity suggests that methanotrophs contribute relatively little to microbial community development during early succession on volcanic deposits.

Materials and Methods

Site description

Volcanic deposits and soils were collected from a number of previously described sites8,21,32 in the vicinity of Kilauea volcano caldera and from two additional sites: An 1855 lava flow on Mauna Loa volcano (ML-1855) that supported a Metrosideros polymorpha (Ohio) forest with a peat-like, acidic (pH 3.5) soil deposit, and a deep (>1 m) soil deposit on a >50,000-yr-old Mauna Kea (MK) lava flow that supported a mixed forest of Acacia koa and various understory species.

Methane uptake assays

Tripletic intact cores were obtained from ML-1855, MK and from a 1959 tephra deposit, 1894 and 1921 lava flows, a 1790 ash deposit, and a forest with mixed Metrosideros polymorpha and (Chain of Craters Road, CCRd, about 300 yr old) on Kilauea volcano during April, 2004. All cores were collected using aluminum tubes (7.4 cm diameter×20 cm length), which contained approximately 10 cm of surface deposits. Atmospheric methane uptake was assayed by sub-sampling at intervals the headspaces of sealed intact cores incubated at ambient temperature (18–22°C). Maximum potential methane uptake capacity at about 100 ppm concentrations was also measured using surface material (0-2 cm) from all sites and sub-surface samples from ML-1855, MK and CCRd. For these assays, about 5 gfw of each deposit were incubated in triplicate in sealed jars (110 cm3) to which methane had been added. Methane concentrations were determined using a Sente Model GS-19S Methane Gas Analyzer equipped with a semi-conductor detector and operated with a zero air carrier gas flow of 20 cm3 min−1. Detector responses were standardized with a known methane concentration of 3.16 ppm for low levels and 100 ppm for elevated levels.

DNA extraction and pmoA-amoA gene amplification

DNA was extracted from triplicate 0.25–0.5 gfw samples of
CCRd, MK and ML-1855 using a MoBio Soil Extraction kit (Carlsbad, CA). Samples were immediately frozen after collection, transported at −80°C and held at −20°C until use. DNA extracts were used in PCR performed with previously described conditions for primers A189/A682 that amplify both pmOA and amoA (ammonia monooxygenase sub-unit A) (31). Amplicons of the correct size from the triplicate samples for each site were pooled then cloned using a TOPO TA kit (Invitrogen, Carlsbad, CA, USA). Inserts were amplified using T3–T7 primers, and then sequenced bi-directionally. Clone sequences were subjected to BLAST analysis to identify those likely represented pmOA or amoA; candidate sequences were then analyzed by PAUP* (31) and Arlequin software (60) to determine phylogenetic relationships and the statistical significance of differences between sites, respectively.

Results and Discussion

Methanotrophic activity

Atmospheric methane consumption was consistently observed at only three sites: CCRd, MK and the ML-1855, and occasionally observed for vegetated tree “islands” and bare tephra on a 1959 deposit, Pu’u Pua‘i; activity was not observed for any other sites, regardless of age. Methane uptake rates varied from 3.8±0.01 μmol CH₄ m⁻² d⁻¹ (0.06±0.01 mg CH₄ m⁻² d⁻¹) for ML-1855 to 114.4±9.4 μmol CH₄ m⁻² d⁻¹ (1.83±0.15 mg CH₄ m⁻² d⁻¹) for CCRd.

Maximum potential uptake rates were highest for sites supporting atmospheric methane uptake. Methane uptake at elevated concentrations was also observed for two sites that did not support atmospheric uptake, but at these sites concentrations decreased slowly from initial values >90 ppm to asymptotes of about 70 and 60 ppm, respectively (not shown). No uptake was observed for other sites.

Maximum potential uptake increased with depth at two sites, CCRd and ML-1855, with highest potentials occurring at 5–10 cm depths (Fig. 1). In contrast, no trend for activity with depth was observed for MK (Fig. 1). Integrated over depth, the highest overall activity was observed for ML-1855.

Atmospheric methane uptake rates for the three consistently active sites fall within the lower range of estimates for continental forests and grasslands (8,17). While the results suggest that methanotrophs likely arrive relatively early (within 40 yr) during succession, their activity is modest at best. Based on comparisons with atmospheric CO and hydrogen uptake (18,21), methanotrophic activity does not appear to contribute significantly to bulk carbon flow. Indeed, atmospheric methane uptake rates are up to 10-fold lower than atmospheric CO and hydrogen uptake. Thus, atmospheric methane likely plays a minimal role in biotic succession on volcanic deposits.

Rather, methanotrophs appear to depend on substantial floral and perhaps microbial community development to condition volcanic materials. This may include development of endogenous sources of methanol, which promotes atmospheric methane uptake (5). However, specific factors that limit methanotroph colonization and development are unknown at present. Understanding these limits, however, may improve prospects for managing continental soils as sinks for atmospheric methane, and for understanding responses of methanotrophs to disturbances, e.g., climate change.

Though atmospheric methane uptake rates are lower than for continental sites, CCRd and ML-1855 depth profiles are similar to those reported for other forests with a sub-surface maximum even though methane concentrations are maximal at the soil surface (19,35). In contrast, maximum CO and hydrogen uptake potentials are greatest in surface soils, where CO concentrations are also greatest (18,23). This suggests that one factor controlling the development of methanotrophic communities and their activity may be soil depth, which is related to basalt weathering rates, plant community development and local climate.

Molecular ecological analyses

pmOA/amoA PCR products were obtained, cloned and sequenced for sites CCRd and MK. No amplicons of the correct size were obtained from ML-1855, in spite repeated attempts using a variety of reaction conditions. Since internal standards (positive controls) proved successful, it appeared that the target genes were not sufficiently abundant or were too divergent in sequence to bind the primers (see below). For the successful amplifications, 204 clone sequences were obtained of which 51 and 113 sequences were affiliated with an amoA clade and a pmOA clade, respectively (Fig. 2, unique sequences only).

Analyses of inferred amino acid sequences revealed the presence of at least 90% of residues considered “universal” signatures for amoA and pmOA (31). Clone sequences clustering with a pmOA clade contained at least 62% of pmOA signatures residues, and <21% of amoA signature residues. In contrast, clone sequences clustering with an amoA clade contained <21% and up to 100% of residues considered signatures for pmOA and amoA, respectively. Clones not affiliated with either clade contained <31% and 35% of the amoA and pmOA signature sequences, respectively.

pmOA sequences derived from the two sites differed distinctly. Mean pairwise differences for CCRd and MK were 14.7±1.0 and 229±11.1, respectively. Nucleotide diversities

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**Fig. 1.** Depth profiles of maximum methane uptake capacity (ng CH₄ gdw⁻¹ h⁻¹) for CCRd forest ( ), Mauna Kea forest ( ) and the 1855 Mauna Loa lava flow ( ). All data are means of triplicates±1 standard error. Depth intervals for samples are indicated by bars on the right.
were higher for MK than CCRd: 0.43±0.21 versus 0.28 ±0.14. AMOVA analysis (analysis of molecular variance) as implemented in Arlequin resulted in an $F_{ST}$ value of 0.261 ($p=0.001$), which was consistent with well-differentiated communities. A plot of cumulative mismatch distribution within each clone library versus mismatch number (Fig. 3) also supports differentiation between the communities. Most of the mismatch among MK clones occurs at a relatively low mismatch level, indicating that the sequences are relatively similar and derived from closely related taxa; the distribution of mismatches for CCRd clones includes a much greater level of mismatch, indicating that sequences are more dissimilar and derived from more distantly related taxa.

A phylogenetic analysis (Fig. 2) also supports differentiation among sites. CCRd was predominantly associated with two clades, one comprised of clones and isolates from tundra soil, and the other associated with a clone (RA14) from a temperate beech forest soil. Though none of the sequences were closely associated with known isolates, most appeared related to "type II" methanotrophs.

In contrast to differentiation of pmoA sequences between sites, no differentiation was evident for amoA-like sequences (not shown). These sequences were most closely related to a sequence from *Nitrosolobus multiformis* (data not shown). In addition to the limited diversity of *amoA* sequences at the two forested sites, neither site supported significant rates of ammonia oxidation (unpublished results). Collectively molecular ecological analyses reveal communities that appear dominated by novel methanotrophs harboring relatively divergent *pmoA* genes that are most closely related to sequences obtained from tundra soils and a Danish beech forest soil. Both of these sites are much older developmentally and are dramatically different climatically than the Hawaiian sites.

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**Fig. 2.** Phylogenetic analysis by neighbor-joining of 197 residues of inferred amino acid sequence for *pmoA* clones from CCRd forest and Mauna Kea forest sites with 1000 bootstrap replicates (bootstrap values >50% indicated at nodes; numbers in parenthesis for methanotroph clones indicate occurrences greater than one). CCRd and Mauna Kea forest clone sequences are deposited as EU723741-EU723759.
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Fig. 3. Mismatch plot (see text) showing cumulative levels of total mismatch within CCRd forest (●) and Mauna Kea forest (○) clone libraries as a function of mismatch level.

Clone library analyses also reveal distinctly different communities for the two Hawaiian sites (Figs. 2 and 3), likely due to differences in deposit age and successional development, as well as the impact of local edaphic factors. How such factors structure methanothroph communities remains uncertain. Likewise, the relationship between community structure and activity is unclear and confounded by higher atmospheric methane uptake rates for CCRd with maximum potential uptake rates greater for MK (Fig. 1).

The lack of success in amplifying pmooA genes for ML-1855 is puzzling, since this site supports significant rates of atmospheric methane uptake as well as some of the highest maximum potential uptake rates observed (Fig. 1). One possibility is that this site supports rather different methanothroph communities. Low pH at ML-1855 (3.5) may select for populations dominated by acidiphilic methanothrophs, such as Methylocella that lack pmoo genes$^{10}$, and for which primer sets different than those used here are necessary.

Collectively, results for recent Hawaiian volcanic deposits reveal patchy distributions of activity occurring most consistently on sites supporting well-developed plant communities. This suggests that methanothrophs may not function as pioneering colonists, but that they require complex systems in which to proliferate. Although not intended to be synoptic, results from pmooA analyses are consistent with other studies$^{14,27,33,39}$ that reveal a limited number of taxa, often including novel lineages. Notably, the extent of diversity for facultative lithotrophs and CO-oxidizing bacteria in Hawaiian volcanic deposits appears much greater than that for methanothrophs$^{11,31}$. Again, this suggests a limited role for methanothrophs in developing Hawaiian ecosystems.

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