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The chloroform fumigation efficiency in water-saturated soils increases by mixing sand and decreasing packing thickness

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ABSTRACT

The chloroform fumigation efficiency for measuring microbial biomass in water-saturated soils is low due to limited diffusion of chloroform. To suggest modifications for the improvement of the fumigation efficiency, the effects of sand mixing and soil packing thickness on the fumigation efficiency in water-saturated soils were investigated through four experiments. Two soils with contrasting organic carbon content were used to examine if the modifications are applicable regardless of microbial biomass level. The soils were waterlogged to mimic rice paddy soils, and sand was mixed to decrease water content by either adding an increasing amount of sand to a decreasing amount of soil (Exp-1) or an increasing amount of sand to a constant amount of soil (Exp-2). Soil packing thickness was decreased by packing a decreasing amount of soil into same-sized containers (Exp-3) or by placing the same amount of soil into progressively wider containers (Exp-4). Overall, the effects of sand mixing and packing thickness on the fumigation efficiency did not differ with soil microbial biomass level. In both soils which were originally saturated with water, increasing the amount of sand mixing decreased ($P < 0.001$) soil water content and increased ($P < 0.001$) the concentration of extracted organic carbon by fumigation (EOC_f) by up to 303% in Exp-1 but decreased ($P < 0.001$) EOC_f by up to 80% in Exp-2, likely due to the concomitant increase in soil packing thickness. Decreasing soil packing thickness increased ($P < 0.001$) EOC_f by up to 128% (Exp-3) and 298% (Exp-4). It was also found that the amount of soil packed affected the fumigation efficiency (Exp-2 and 3). We suggest using the same amount of sample and same-sized beakers for fumigation is more practical to minimize the confounding effect of soil amount and packing thickness on the fumigation efficiency of water saturated soils and thus to help the comparison of microbial biomass between soils.

1. Introduction

Soil microbes directly affect soil ecosystem stability and fertility through nutrient cycling and organic matter decomposition in the soil [1]. As the extent of the nutrient and carbon (C) turnover by microbes is directly linked to the size of the microbial populations, the measurement of microbial population is a basic procedure that is required for the estimation of soil quality [2–4].

One of the methods used to quantify the microbial population size is the measurement of microbial biomass in the soil [3,5]. Several methods are available to measure soil microbial biomass including chloroform ($CHCl_3$) fumigation-incubation (CFI) [6], $CHCl_3$ fumigation-extraction (CFE) [7], substrate induced respiration (SIR) [8], and measurement of the concentration of cellular compounds such as adenosintriphosphate (ATP), phospholipid fatty acids (PLFA), and double stranded deoxyribonucleic acids (dsDNA) [9,10]. Each method has its advantages and disadvantages [3,5]; e.g., the procedure of CFI is simple but is not applicable to soils containing substantial amount of easily decomposable C sources, the CFE overcomes the limitation of the CFI but the conversion factor (k_{EC}) is still controversial, the SIR method requires an intensive measurement of soil respiration and is also susceptible to

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organic soil amendment. Though the measurement of ATP, PLFA, and dsDNA are targeted to living microorganisms and thus can be more precise than the others, the extraction and measurement procedures are more complicated than other methods [5,10]. Among the methods, the CFE method is widely used particularly when both microbial biomass C (MBC) and the content of other elements such as nitrogen (N), sulfur, and phosphorus in the microbial biomass need to be determined [1,3,5].

The $CHCl_3$ fumigation method, however, suffers from a low fumigation efficiency for water-saturated soils such as water-logged paddy [11,12] and peatland soils [13] due to restricted permeation of the hydrophobic $CHCl_3$ through water-filled soil pores [14], resulting in the underestimation of microbial biomass [15,16]. However, factors affecting the fumigation efficiency in water-saturated soils have not been systematically studied. To overcome the low fumigation efficiency for water-saturated soils, a few modifications that can facilitate $CHCl_3$ contact with soil particles such as direct addition of $CHCl_3$ to soils [12,14,17,18] and spreading soils in thin layers [11,19] have been proposed. However, direct addition of $CHCl_3$ to the soils may cause overestimation of microbial biomass due to the greater adsorption of $CHCl_3$ molecules onto soil particles as compared with the standard fumigation method where the $CHCl_3$ vapor is diffused into the soil [20,21]. Although spreading soils in thin layers can facilitate $CHCl_3$ contact with soil particles, the effect of soil packing thickness on the fumigation efficiency has not been studied for water-saturated soils. Therefore, an assessment of the relationship between soil packing thickness and the fumigation efficiency in water saturated soils may help us improve the microbial biomass measurement using $CHCl_3$ fumigation for such soils.

In a previous study [15], we reported that mixing sand into a water-saturated soil has been found to decrease water content and thus improve the fumigation efficiency. However, in that study [15], not only soil water content but also soil packing thickness decreased as sand mixing increased bulk density of the soil-sand mixture. Therefore, we still do not understand clearly how sand mixing affected (water content vs. packing thickness) the fumigation efficiency in water-saturated soils. This study was conducted to understand the effect of two key factors (water content and soil packing thickness) on the $CHCl_3$ fumigation efficiency for water-saturated soils and to suggest ways to improve the fumigation efficiency for microbial biomass measurement. Specifically, we investigated the effect of different ratios of sand mixing with water-saturated soil and different soil packing thickness in the beaker used for soil fumigation on the fumigation efficiency for water-saturated soils. The hypotheses tested in this study were 1) mixing sand with water-saturated soil increases the fumigation efficiency by reducing soil water content or increasing air-filled pores for $CHCl_3$ diffusion, 2) decreasing soil packing thickness improves the fumigation efficiency due to reduced path for $CHCl_3$ diffusion into the soil, and 3) the effects of sand mixing on increasing the fumigation efficiency may be affected by soil packing thickness; i.e., sand mixing effects may not be apparent if soil packing thickness increases by sand mixing.

2. Materials and methods

2.1. Experimental materials

Soil samples were collected from a paddy field in the experimental farm (126°36′08″E, 35°10′21″N) of Chonnam National University, South Korea. The paddy field has been cultivated for rice since 1960s. The soil was classified as an Inceptisol (coarse loamy, mixed, nonacid, mesic family of Fluvaquentic Endoaquepts) in USDA Soil Taxonomy. This soil type accounts for about 50% of rice paddy area in South Korea [22]. Soil samples (around 5 kg) were collected from five randomly selected points in a plot (10 m × 10 m) of the field during the mid-drainage period in July 2013. The soil samples were air-dried, passed through a 2-mm sieve, and mixed thoroughly in a 10-L plastic container to obtain a homogenous sample for the experiment. A subsample of the soil samples (<2 mm) was used for oven-dried water content measurement and chemical analysis including pH (6.10) at 1-to-5 ratio of sample-to-water (w:v), total C (11.6 g C kg^{-1}) and N (0.92 g N kg^{-1}) with an elemental analyzer (FLASH EA-1112, Thermo Fisher Scientific Inc., Waltham, MA, USA) [23], and soil particle-size distribution (sand, silt and clay at 41.8, 31.7 and 26.5%, respectively) by the pipette method [24]. Chemical grade sand (ranging from 0.3 to 0.6 mm in diameter) was purchased from Wako Pure Chemical Industries (Osaka, Japan) and used after washing with distilled water to remove trace organic C. The dissolved C in the running water was determined using a total organic C analyzer (Sievers 5310 C, GE Analytical Instruments, Boulder, CO, USA), and no trace organic C was detected at the first washing. The washed sand was oven-dried at 105 °C for 24 h and used for the experiments.

In order to compare the effects of sand mixing and soil packing thickness on the fumigation efficiency in soils with different organic C concentration, we prepared another soil by adding 78.7 g of livestock manure compost to the soil (1 kg) described above to increase the soil organic C three-fold (to 34.8 g C kg^{-1}); thus two soils with different soil organic C (and thus different microbial biomass level) concentration were prepared. The compost were analyzed with the same method used for soil analysis and had a pH of 7.38, total C of 295.0 g C kg^{-1}, and total N of 27.5 g N kg^{-1}. The original soil with low soil organic C and the compost-amended soil with high soil organic C are referred to below as S_{LOC} and S_{HOC}, respectively.

One kilogram of the soil was placed into a plastic container (14.8 × 20.8 × 15.0 cm). Distilled water (totally ca. 500 mL) was added to the soil and mixed with a spatula, and the procedure was repeated until the soil glistens as it reflects light, indicating that the soil was saturated with water, following the process in Rhoades [25]. Additional (154 mL) distilled water was added to create a water table of 0.5 cm above the soil surface to mimic paddy-like conditions. The soils were incubated at 25 ± 1 °C in the dark for 7 days to allow microbial population growth and were then used for the following fumigation experiments. The oven-dried sand and the distilled water used were tested for microbial contamination with the spread plate technique using Lysogeny broth (LB) agar plate [26] and found no microbial colony, indicating the sand and distilled water are microbe-free (see supplementary content).

2.2. Experimental design and treatments

Four experiments (two on sand mixing and two on packing thickness) were sequentially conducted with the two soils with three replications (Fig. 1). In experiment 1 (Exp-1), increasing amounts (0, 1, 2, 4, 5, 6, 7, and 8 g) of sand was mixed with correspondingly decreasing amount (10, 9, 8, 6, 5, 4, 3, and 2 g) of soils, resulting in a constant amount (10 g) of soil-sand mixture in 100 mL beakers (Fig. 1a). This resulted in a decrease in soil water content (from 0.53 to 0.11 cm cm^{-3}) and a slight decrease in the packing thickness (from 0.90 to 0.68 cm) of the soil-sand mixture (Table 1). In experiment 2 (Exp-2), increasing amounts of sand (0, 2, 4, 5, 6, 7, 8, and 10 g) were mixed with a constant amount (10 g) of soil in 100 mL beakers (Fig. 1b), resulting in a decrease in soil water content (from 0.53 to 0.28 cm cm^{-3}) and an increasing volume and thickness (from 0.90 to 1.73 cm) of the soil-sand mixture (Table 1). The range of sand mixing rate was narrower in Exp-2 (0.0–50.0%) than in Exp-1 (0.0–80.0%) as we intended not to let the total