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Chloroform fumigation extraction for measuring soil microbial biomass: The validity of using samples approaching water saturation

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ABSTRACT

The chloroform fumigation-extraction method for determining soil microbial biomass is commonly applied at soil moisture (SM) levels of 40–50% water-holding capacity (WHC). Below that moisture range, restricted enzymatic autolysis might limit the fumigation flush of extractable cellular material, thus underestimating microbial biomass. Likewise, the upper range of SM (i.e., > 50% WHC) is considered problematic due to an alleged reduced fumigation efficiency when vapor diffusivity through soil is reduced. The current investigation demonstrated relatively constant fumigation flushes along a 40–90% WHC moisture gradient alongside unchanging carbon-to-nitrogen flush ratios corresponding to characteristic cellular values. This clearly refuted the proposed reduced fumigation efficiency under elevated moisture conditions, and thus validated the applicability of the method to samples collected moist.

1. Background

The chloroform fumigation extraction (CFE) procedure for determining soil microbial biomass prescribes a soil moisture (SM) level of 40–50% water-holding capacity (WHC) (Grace et al., 2006; Vance et al., 1987). This specification was supported by maximal extractable organic-carbon (OC) or nitrogen (N) flush values obtained under SM conditions ranging from 30% to 63% WHC (Amato and Ladd, 1994; Davidson et al., 1989; Ross, 1988, 1989; Sparling and West, 1989; Sparling et al., 1990). Reduced flushes were recorded at ~20% and, particularly, below 10% WHC (Sparling and West, 1989). Still, higher moisture levels have never been reported to reduce the flush (e.g., Amato and Ladd, 1994; Davidson et al., 1989). Therefore, the goal of the study was to systematically determine the effect of SM maintained during fumigation on both the extractable-OC and -N flushes, from relatively dry field conditions toward soil saturation, using contrasting soil types. This allowed assessing the validity of extending the method's application to samples collected in relatively wet states, e.g., recently rain-fed or irrigated soil.

2. Experimental

Fumigation-extractable OC data obtained for a range of soil properties and SM conditions (Rotbart et al., 2017), were integrated with new data on extractable N for the mutual analysis of OC and N fumigation flush response patterns to SM variation. In brief, long-term differential fertilization (urea versus compost application at a rate of 60 m^3 ha^{-1} yr^{-1}) taking place in two distinct climatic regions and soil types (Vertisol, Newe Ya'ar (NY) Research Center, Northern Israel; Loess, Gilat (GIL) Research Center, Southern Israel) has resulted in adjacent plots having the same soil type but different soil organic-matter (SOM) contents. Thus, four soil treatments were obtained, referred to as NY COM 0 (i.e., urea fertilization), NY COM 60 (i.e., compost application), GIL COM 0, and GIL COM 60. The NY and GIL soils differed in mechanical composition (64 versus 25% clay, respectively) and in total OC content (1.62, 2.27, 0.87, and 1.33% for NY COM 0, NY COM 60, GIL COM 0, and GIL COM 60 soils, respectively). SM was adjusted immediately before fumigation to either ~20 (level at sampling), 30, 40, 50, 60, 70, 80, or 90% WHC. CFE and measurements of OC in fumigated and non-fumigated soil extracts were performed

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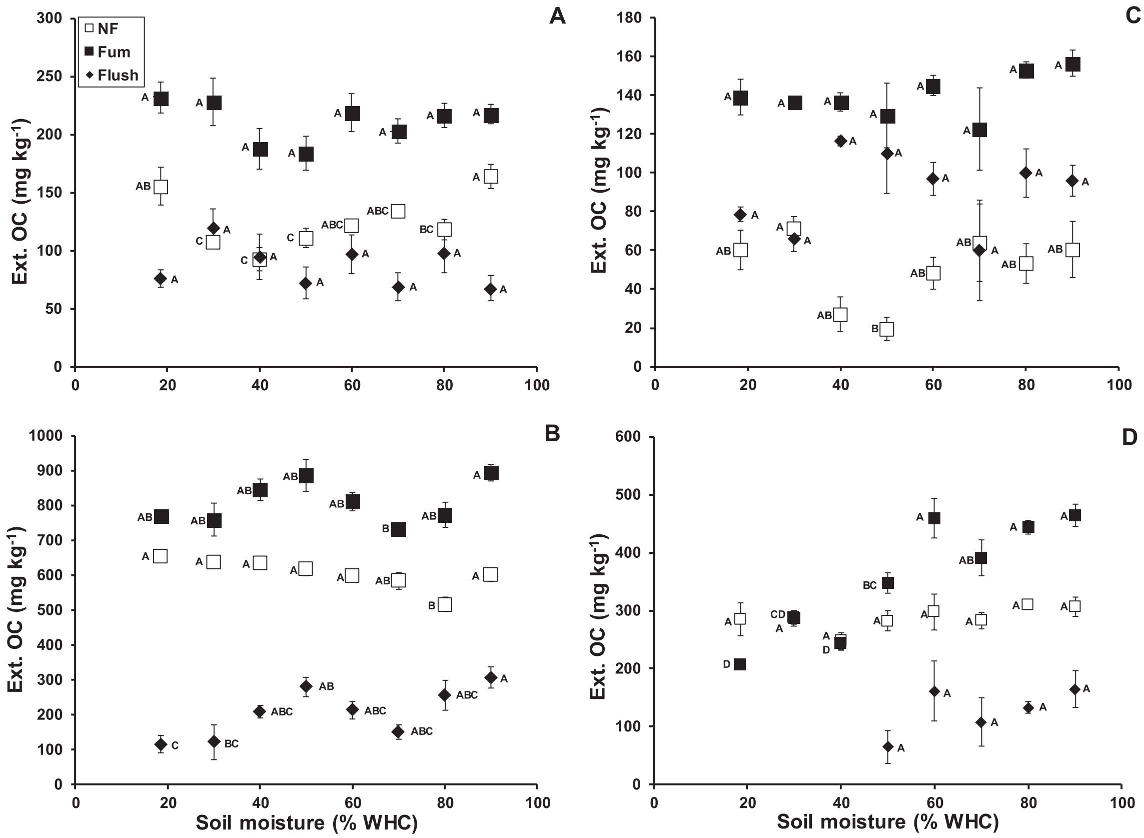

Fig. 1. 0.5 M K_2SO_4-extractable organic carbon (abbreviated Ext. OC) concentrations with respect to non-fumigated (abbreviated NF) and fumigated (abbreviated Fum) soil extracts and flush, as a function of soil moisture maintained during fumigation (expressed as percentage of soil water holding capacity). Error bars indicate the standard errors of the means. Different letters beside the value symbols denote significant differences between the means along the soil moisture gradient ($P < .05$, Tukey–Kramer) tested by one-way ANOVA for each treatment (combination of soil and compost level), using JMP 12.0.01 (SAS). The four investigated soil varieties were designated letters A–D: (A; top-left) NY COM 0; (B; bottom-left) NY COM 60; (C; top-right) GIL COM 0; and (D; bottom-right) GIL COM 60. The data for the figure were adopted from Rotbart et al. (2017; Table 2).

following Vance et al. (1987), with minor modifications detailed by Rotbart et al. (2017). Extractable ninhydrin-reactive-N (Nin-N) was determined spectrophotometrically following Joergensen and Brookes (1990) using leucine standards and absorbance at 570 nm. Extractable-NH_4^+-N was determined using an autoanalyzer (Quickchem 8000, Lachat Instruments). Subtracting the values obtained for non-fumigated sample extracts from those obtained for corresponding fumigated sample extracts provided the extractable flushes.

3. Influence of SM on OC and N fumigation flushes

Wetting soil from 20% to 30–90% WHC generally increased fumigation flushes of OC, Nin-N, and NH_4^+-N (Figs. 1–3). Changes in the OC flush varied (i.e., across the 30–90% WHC range) between −24 and +57% (mean 16%) for the urea-fertilized NY and GIL soils (i.e., COM 0 treatments) and between +6 and +166% (mean 90%) for the NY COM 60 soil (Fig. 1). For the GIL COM 60 soil, a negative flush at 20% WHC (i.e., non-fumigated > fumigated soil extract OC) and a zero flush at 30–40% WHC did not permit this calculation. For all soils, increasing SM in the range of 50–90% WHC did not significantly affect the OC flush. The scales of change in flush are comparable to previous reports, e.g., Ross (1989), with 15%-lower OC flushes for silt loams fumigated at their summer-moisture level (i.e., 15–30% WHC), relative to the maximal values obtained for samples wetted to 50% WHC.

As with OC, the wetting-induced increases in the Nin-N flushes largely ceased above 50% WHC. Increases were in the range of 128–546% for the NY and GIL COM 0 soils, averaging 410 and 358%, respectively. For the compost-treated soils, the increases were in the range of 429–888% (mean 664%) for the NY COM 60 soil and 88–156% (mean 123%) for the GIL COM 60 soil. These trends are in line with the limited available data on the N fumigation flush response to SM variation. Davidson et al. (1989) reported an increase of ~200% in N flush from a silt loam when SM was increased from 7% to 30, 50, or 70% WHC. Amato and Ladd (1994) found that the wetting of air-dried vertisol and alfisol samples to 20% WHC increased Nin-N flush by 133% and 486%, respectively. Further wetting to 40% WHC resulted in further increases by 416 and 12%, respectively. Elevating the SM to 100% WHC resulted in additional minor flush increases.

Patterns of SM influence on extractable NH_4^+-N flushes (Fig. 3) largely paralleled those for Nin-N. The NH_4^+-N fraction of the Nin-N flush, calculated for the various soils (18–44%; means for the range 40–90% WHC) fell around the mean values of 29% reported by Joergensen (1996) and 30% by Witt et al. (2000). A relative constancy of the NH_4^+-N fraction with SM variation might reflect the lack of moisture influence on the autolysis-mineralization sequence (Joergensen and Brookes, 1990).