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Assessment of Methods for Measuring Soil Microbial Biomass Carbon in Temperate Fruit Tree-Based Ecosystems

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ABSTRACT

We investigated the potential of three methods of quantifying microbial biomass carbon (MBC), viz., chloroform fumigation-extraction (CFE) following organic C estimation through Vance method (CFE-V) and Snyder–Trofymow method (CFE-ST), and substrate-induced respiration (SIR) method in soils under various temperate fruit crops along with a control (no plantation) at 0–20 and 21–40 cm soil depths. CFE methods have shown significant ($p < 0.05$) increase in chloroform labile C in all orchards over the control in surface soil. The interaction between the fruit crops and methods, although significant ($p < 0.01$), indicated that CFE-ST and SIR methods were statistically at par with each other within the same fruit crop, except peach plantation (CEF-ST significantly lower than SIR) in 0–20 cm soil depth. The coefficient of variation recorded for chloroform labile organic C estimates by CFE-ST method makes it more precise than CFE-V method, especially in 0–20 cm soil depth. The very close agreement between the methods suggests that over this narrower range (i.e., smaller geographical area) all methods are appropriate for assessing MBC. However, SIR, being most sensitive to orchard plantations and strongly correlated with various soil chemical properties, could preferably be recommended for estimation of MBC in such soils. As an alternative to CFE-V method, CFE-ST may also be used for estimation of chloroform labile organic C in these soils.

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Microbial biomass C; methods; soil depth; temperate fruit crops

Introduction

Microbial biomass carbon (MBC) is relatively difficult to measure, and it is largely through agreements among different methods that we gain confidence in new measurements (Martens 1995). In comparison to microbial activity, only a few methods are employed for measuring MBC, for example, chloroform fumigation-extraction (CFE) (Vance, Brookes, and Jenkinson 1987), chloroform fumigation-incubation (Jenkinson and Powlson 1976a), substrate-induced respiration (SIR) (Anderson and Domsch 1978), and phospholipid fatty acid analysis (Frostegård, Tunlid, and Bååth 1991). Among these, the CFE method (Vance, Brookes, and Jenkinson 1987) is most widely used by the researchers mainly due to its simplicity and ease of procedure. In this method, soils are exposed to chloroform vapor for 24 h or longer to lyse the microbial cells for releasing chloroform labile organic C (Jenkinson and Powlson 1976b). Then, the fumigated and non-fumigated samples are extracted with 0.5 M K_2SO_4 and organic C in the extract is estimated by acid-dichromate oxidation, and the difference between them is accounted for MBC. This method, therefore, enables a direct measurement of C and other nutrients (e.g., N, P, and S) contained within soil microbial biomass. Nevertheless, tediousness in its procedure (i.e., 30 min reflux digestion following colorimetric titration) makes this method difficult for its use with large number of samples

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within a short period. Moreover, MBC measured by this method may, however, be hampered by large amounts of non-biomass soil organic matter (plant residues and roots) made extractable by K_2SO_4 and, thus could lead to an overestimation of microbial biomass C (Martens 1995).

In recent time, researchers have used the method of total soil and plant C estimation by Snyder and Trofymow (1984) in estimating organic C content in K_2SO_4 extract of both fumigated and non-fumigated soil samples for determination of microbial biomass C (Bhaduri and Purakayastha 2014; Bhaduri et al. 2014; Debnath et al. 2015). This method is same as the fumigation-extraction method up to extraction of chloroform labile C with K_2SO_4, and the only difference lies in the C determination procedure. The organic C in the extract is determined by wet oxidation with mineral acid at a specific temperature that liberates CO_2, which is trapped in a standard alkali solution and quantified titrimetrically. This method is simple, accurate, and can be applicable to a variety of soil types. The advantage of this method is that large number of samples can be processed in a day as compared to acid-dichromate oxidation. Nevertheless, the feasibility and reliability of this method in measuring chloroform labile organic C in K_2SO_4 extract vis-à-vis microbial biomass C remains to be tested. In addition, this method has not been correlated with most widely used acid-dichromate oxidation method (Vance, Brookes, and Jenkinson 1987) till date.

On the contrary, the SIR method is a simple, rapid, and economical method to determine MBC in soils and residues (Bailey, Bolton, and Smith 2008). This method allows estimation of the amount of carbon held in non-resting, living microorganisms in soil sample. The initial respiratory response to added energy-yielding substrate recorded before any development in existing soil microflora could be viewed as an index of the existing soil microflora (Anderson and Domsch 1978). The SIR method has been given significant attention since its development, and it continues to be used, tested, and modified into the twenty-first century (Bailey, Bolton, and Smith 2008). However, this method is also not beyond criticism and has been criticized for its reliance on glucose-utilizing organisms to determine the entire broad spectrum of soil microbial biomass.

Comparison of CFE and SIR methods has been made on many instances in the past. However, such comparisons were arrived from soils with a very wide range of properties and/or over wider geographical areas (Anderson and Joergensen 1997; Setia, Verma, and Marschner 2012). Nevertheless, their comparison over a much narrower area, i.e., between adjacent fields having contrast nature of different temperate fruit crops, has not been critically assessed. Characteristics of the vegetation on a site could influence the composition and functioning of the soil microbial community through alteration of microclimate like shading and uptake/transpiration of soil water, production of root exudates, and interactions with the rhizospheric microorganisms (Prescott and Grayston 2013). In a recent study, Debnath et al. (2015) reported that microbial biomass and other soil biological properties were strongly influenced by the orchards having different types of temperate fruit crops. Thus, the hypothesis of our study was that various orchard plantations might influence the soil organic carbon dynamics due to dissimilar root biomass impacting root exudation behavior and litter fall. We wanted to test this hypothesis through measuring microbial biomass C by a sensitive method. Additionally, we intended to test whether chloroform labile C determined by acid-dichromate oxidation method (Vance, Brookes, and Jenkinson 1987) be comparable with wet oxidation-diffusion method of Snyder and Trofymow (1984). Thus, the objective of this study was (i) to assess the variability among three methods for estimating microbial biomass C in soil under different temperate fruit crops and (ii) to work out the relationships between MBC measured by different methods with various soil chemical properties.

Materials and methods

Study site

The study site is located at the ICAR-Central Institute of Temperate Horticulture (CITH), Srinagar, India (34°05′ N latitude and 74°50′ E longitude; 1640 m msl). This area falls under semiarid temperate region, having cold and chilly conditions from November to February, with an average annual rainfall

range from 600 to 800 mm. The experimental orchards within the study site were planted with apricot (cv. Harcot), peach (cv. Fantasia), plum (cv. Santa Rosa), and cherry (cv. CITH Cherry 16) in between the years 2002 and 2003 and distributed in an area of half a hectare (100 × 50 m) for each. We have also selected a control plot (no plantation) of same size, comprising mainly perennial grasses that has been a fallow land from the past 15 years and has never been fertilized. The fertilizer doses applied to the fruit crops are 450 g N $tree^{-1}$ for apricot, peach, and plum and 300 g N $tree^{-1}$ for cherry; 150 g P_2O_5 $tree^{-1}$ for apricot, plum, and cherry and 390 g P_2O_5 $tree^{-1}$ for peach; 750 g K_2O $tree^{-1}$ for apricot, peach, and plum and 440 g K_2O $tree^{-1}$ for cherry. One-fourth of fertilizer dose is applied to all fruit crops in the first week of February before blossoming and half dose of fertilizer is applied 15 days after first split. The rest is applied 25 days after second split. Farmyard manure at the rate of 20 kg $tree^{-1}$ is added to each fruit crop during second fortnight of December.

Soil sampling

Soil sampling was performed during second week of May in 2013 and 2014 within the rhizhospheric zone of the selected fruit crops at two depths, viz., surface (0–20 cm) and subsurface (21–40 cm). At sampling, all fruit crops were at fruit setting stage, which is considered as an active phase of growth. Subsurface sampling was performed mainly due to the fact that more than 90% of roots were distributed within 45 cm. Therefore, it can be expected that microbial activities would be affected along the depth due to rhizospheric activities. A total of 30 soil samples (5 orchards × 2 depths × 3 replications) were collected, sieved (2 mm mesh) to remove discrete plant tissues, and then placed in labeled plastic bags and kept at 4 °C until further analysis. The gravimetric moisture content in samples was determined immediately. A subset of soil samples was air dried and passed through a 2 mm sieve for determination of pH (1:2 soil: water ratio) (Jackson 1967), organic C (Walkley and Black 1934), available N (Subbiah and Asija 1956), available P (Olsen et al. 1954), and available K (Schollenberger and Simon 1945) (Table 1). The soil is Inceptisol and classified as *Typic Haplustept* (USDA classification), and sandy loam in texture with sand 65%, silt 22%, and clay 13%.

Measurement of microbial biomass C

CFE method

Moist sample was taken in duplicate (to give approximately 10 g oven-dry weight) in 50 mL glass beakers. One set was kept inside a vacuum desiccator and fumigated with fresh ethanol-free chloroform for 24 h in the dark (Jenkinson and Powlson 1976b). Both fumigated and non-fumigated subsamples were extracted with freshly prepared 0.5 M K_2SO_4 for 30 min and filtered to get extract. The following methods were used for determination of organic C in extracts.

Vance method (CFE-V)

Organic C in the filtered K_2SO_4 extracts (1:4 w/v) was measured by acid-dichromate oxidation (Vance, Brookes, and Jenkinson 1987). The additional oxidizable C obtained from the fumigated soils were taken to represent the microbial C flush and converted to MBC using the relationship:

$$\text{Microbial biomass carbon} = (OC_f - OC_{uf})/0.45 \quad (1)$$

where OC_f and OC_{uf} are chloroform labile organic carbon extracted from fumigated and non-fumigated soil, respectively.

Snyder–Trofymow method (CFE-ST)

Organic C in K_2SO_4 extracts (1:2.5 w/v) was determined by wet oxidation diffusion method (Snyder and Trofymow 1984). Also, 5 mL of extract was transferred to diffusion tube and digested in the presence of potassium persulfate ($K_2S_2O_8$) and 0.025 M H_2SO_4 in a digestion block at 120 °C for 2 h. The amount of CO_2-C evolved was trapped in a shell vial containing 4 mL of 0.1 N NaOH kept over the

Table 1. Properties of air-dried soil under different temperate fruit crops at the experimental site.

Fruit crops	Soil property	Soil depth (cm) 0–20	Soil depth (cm) 21–40	Between layers†	Soil property	Soil depth (cm) 0–20	Soil depth (cm) 21–40	Between layers†
	pH				Available N (mg kg^{-1})			
Control		6.93 ± 0.10^{ab}	7.14 ± 0.11^{a}	ns		96.7 ± 9.7^{a}	68.2 ± 9.3^{a}	ns
Apricot		6.70 ± 0.11^{a}	7.16 ± 0.08^{a}	ns		134.5 ± 9.7^{ab}	97.5 ± 10.9^{a}	*
Plum		7.18 ± 0.03^{b}	7.27 ± 0.02^{a}	ns		118.9 ± 7.5^{ab}	82.8 ± 10.1^{a}	ns
Peach		7.10 ± 0.05^{b}	7.33 ± 0.05^{a}	ns		148.6 ± 10.5^{b}	80.0 ± 6.8^{a}	*
Cherry		7.02 ± 0.09^{b}	7.21 ± 0.08^{a}	ns		138.3 ± 15.1^{b}	82.5 ± 9.7^{a}	*
Mean		6.99 ± 0.07	7.22 ± 0.08			127.2 ± 10.7	82.3 ± 9.3	
	Organic C (g kg^{-1})				Available P (mg kg^{-1})			
Control		8.7 ± 0.1^{a}	4.9 ± 0.1^{a}	*		23.3 ± 3.4^{a}	17.4 ± 3.5^{a}	ns
Apricot		11.5 ± 0.1^{b}	7.0 ± 0.1^{ab}	*		31.3 ± 4.7^{a}	22.4 ± 3.3^{a}	*
Plum		11.8 ± 0.1^{ab}	9.1 ± 0.1^{b}	ns		26.8 ± 4.0^{a}	19.9 ± 2.1^{a}	*
Peach		13.6 ± 0.1^{b}	8.0 ± 0.1^{b}	*		29.7 ± 4.0^{a}	23.1 ± 5.4^{a}	*
Cherry		10.7 ± 0.1^{ab}	7.3 ± 0.1^{ab}	*		27.0 ± 5.6^{a}	20.7 ± 3.7^{a}	*
Mean		11.3 ± 0.1	7.3 ± 0.1			27.7 ± 4.4	20.7 ± 3.8	
	Moisture (mg kg^{-1})				Available K (mg kg^{-1})			
Control		128.0 ± 2.8^{a}	143.7 ± 1.6^{a}	ns		49.3 ± 3.7^{a}	35.4 ± 0.8^{a}	ns
Apricot		171.8 ± 1.8^{b}	182.2 ± 2.5^{b}	ns		76.2 ± 5.7^{c}	53.7 ± 3.1^{b}	*
Plum		176.2 ± 12.3^{b}	188.5 ± 2.3^{b}	ns		54.6 ± 0.9^{a}	40.6 ± 2.6^{a}	ns
Peach		198.3 ± 2.3^{c}	209.6 ± 5.0^{c}	ns		63.3 ± 9.0^{ab}	41.7 ± 4.0^{a}	*
Cherry		165.9 ± 3.4^{b}	186.4 ± 5.1^{b}	*		68.9 ± 5.5^{b}	42.1 ± 2.0^{a}	*
Mean		168.0 ± 4.6	182.1 ± 3.5			62.5 ± 4.8	42.7 ± 2.4	

Values are means (n = 3) ± SE.
Values followed by different alphabets in superscript are significantly different at $p < 0.05$ based on Duncan's multiple-range test (DMRT).
ns, nonsignificant.
† Significance between the soil layers of same fruit crop at $p < 0.05$.

indentation inside diffusion tube. After digestion, the diffusion tube was allowed to remain undisturbed for 12 h to ensure complete absorption of evolved CO_2-C. After 12 h, the shell vial was taken out and the unspent alkali was titrated against 0.02 N HCl in the presence of an excess of 1 M $BaCl_2$ to stabilize the trapped CO_2-C. Chloroform labile C extracted was converted to MBC using the following relationship:

$$\text{Microbial biomass carbon} = (OC_f - OC_{uf})/0.45 \quad (2)$$

SIR method

Soil microbial biomass C was estimated by the SIR method (Bailey, Bolton, and Smith 2008). The method involved the use of moist soil (equivalent to 10 g oven-dried soil) weighed into 40 mL tubes and amended with 10 mg glucose solution (1% w/v) and mixed thoroughly. The tubes were then sealed with air-tight rubber septum and incubated for 1 h at room temperature. After 1 h, the CO_2 trapped in the headspace was sampled (0.5 mL) with a syringe and measured by a gas chromatogram (GC-4890D, Agilent Technologies Inc., Santa Clara, USA). The CO_2 flush (mL kg soil^{-1} h^{-1}) generated during a predetermined incubation period is correlated to biomass C (mg kg soil^{-1}) as shown in Eq. (3) (Anderson and Domsch 1978).

$$x = 40.04y + 0.37 \quad (3)$$

where x is the microbial biomass C (mg kg^{-1} soil) and yw is the rate of CO_2 evolution (mL CO_2 kg^{-1} soil h^{-1}).

Statistical analysis

Duncan's multiple-range test (DMRT) and least significant difference at $p < 0.05$ for comparison of significant differences between means have been performed using SPSS 16.0 (SPSS Inc., Chicago,

USA). A two-way analysis of variance was performed to elucidate the effects of different temperate fruit crops, microbial biomass C measured with three methods, and their interactions on microbial biomass C at two soil depths. Multiple linear regression analysis was performed with microbial biomass C measured by the three methods as dependent variables and some selected soil properties as independent variables to identify the factor contributing most significantly. Multivariate correlation matrix (Pearson) was also worked out between soil chemical properties and three methods of biomass C estimation to show their degree of associations.

Results and discussion

Chloroform labile C estimated by CFE-ST and CFE-V methods in the surface soil (0–20 cm) varied from 457 to 1000 and 636 to 1198 mg kg^{-1} soil, respectively (Table 2). While MBC ranged from 517 to 1064 mg kg^{-1} soil as measured with the SIR method. The same in the subsurface soil (21–40 cm) varied between 307 and 692 mg kg^{-1} soil by the CFE-ST method, 586 to 729 mg kg^{-1} soil by the CFE-V method, and 242 to 393 mg kg^{-1} soil by the SIR method. In surface soil, the methods varied among themselves regarding ranking (highest to lowest value) of the selected orchards. However, CFE methods and SIR method ranked the selected orchard soils in exactly the same order (plum > peach > apricot > cherry > control) in subsurface soil. Further, the CFE-ST method produced results, which ranked the selected orchards in same order in both soil depths. The three methods have shown significant increase in the said parameter in all orchards compared to the control in surface soil. However, the same is not true in subsurface soil, except CFE-V. In majority, the orchards did not vary significantly with each other (excluding control) in both the soil depths in terms of measured MBC, while peach and plum orchards varied significantly from each other in the SIR method in surface soil. Nonetheless, chloroform labile C was found to be significant between the soil layers of same types of fruit crop as estimated by CFE-ST and CFE-V. Similar observation was also recorded in MBC estimated by the SIR method. It suggests that microbial biomass in surface soil was perhaps influenced by the inputs added as well as litter fall, whereas root exudates and other root-related activities were probably the principal governor of microbial biomass in subsurface soil.

Chloroform labile C must be converted to MBC using an efficiency factor (0.45), which corrects the incomplete extraction (Wu et al. 1990). It suggests that 45% of total C in the cells of microbial biomass is rendered extractable by K_2SO_4, following 24 h chloroform fumigation. It is to be noted that we have used a ratio (soil:K_2SO_4) of 1:4 in the CFE-V method and 1:2.5 in the CFE-ST method. Higher chloroform labile C was obtained in the method with wider ratio. In other words, the extraction efficiency of K_2SO_4 was lower in the CFE-ST method, and therefore, a different efficiency factor should

Table 2. Effect of interaction between the type of fruit crops and chloroform labile C or biomass C measured with the three methods.

	Chloroform labile or biomass C (mg kg^{-1})								
	Method						Significance between soil layer†		
	CFE-ST	CFE-V	SIR	CFE-ST	CFE-V	SIR	CFE-ST	CFE-V	SIR
Fruit crops	0–20 cm			21–40 cm					
Control	457 ± 32^{bA}§	636 ± 73^{bA}	517 ± 36^{cA}	307 ± 29^{aB}	586 ± 53^{aA}	242 ± 32^{aB}	ns	ns	*
Apricot	852 ± 80^{aB}	1198 ± 32^{aA}	975 ± 66^{abB}	592 ± 70^{aA}	700 ± 20^{aA}	386 ± 12^{aB}	*	*	*
Plum	1000 ± 80^{aA}	1194 ± 9^{aA}	783 ± 152^{bB}	692 ± 4^{aA}	729 ± 16^{aA}	393 ± 22^{aB}	*	*	*
Peach	928 ± 38^{aB}	1064 ± 178^{aA}	1064 ± 26^{aA}	684 ± 63^{aA}	728 ± 9^{aA}	390 ± 12^{aB}	*	*	*
Cherry	825 ± 93^{aA}	932 ± 84^{aA}	834 ± 35^{abA}	513 ± 34^{aA}	660 ± 79^{aA}	350 ± 44^{aB}	*	*	*
Mean	812 ± 65	1005 ± 75	835 ± 63	558 ± 40	680 ± 35	352 ± 24			

Values are means (n = 3) ± SE and expressed as mg kg^{-1}.

CFE-V, chloroform labile C measured with Vance method; CFE-ST, chloroform labile C measured with Snyder–Trofymow method; SIR, biomass C measured with substrate-induced respiration method; ns, nonsignificant.

§Values followed by lowercase letters in superscript in a column (orchard) and capital letters in a row (method) in a particular soil depth are significant according to Duncan's multiple-range test (DMRT) at $p < 0.05$.

†Significance between soil layer of same fruit crop at $p < 0.05$.

have been used to convert chloroform labile C into MBC. This could probably give an indication that the efficiency factor used was not the same for both the CFE methods, which is further supported by the differences in mean MBC estimates by those methods in surface and subsurface soil. A range of efficiency factor (0.38–0.58) is proposed by many researchers in the past, and it has not been tested which is best suited for the soils used here, and consequently, appropriate conversion values need to be assessed and used for converting the data obtained into MBC. Chloroform labile C estimates of the orchard soils were lower with the CFE-ST method (where a smaller soil:extractant ratio was used). It implies that the CFE method is affected by the ratio of soil to extractant. Therefore, while measuring chloroform labile C by the CFE-ST method a wider ratio is recommended, particularly in soils with high organic C content, because water-holding capacity and thus the soil moisture content depend on the organic C content (Beck et al. 1997).

The main effect of the methods showed CFE-V to estimate significantly higher chloroform labile C than CFE-ST (Table 2); however, they were statistically at par with each other (except control) at 21–40 cm soil depth, meaning that they are comparable in subsurface soil. The apricot and peach orchards clearly showed significantly higher extracted chloroform labile C by the CFE-V than the CFE-ST method at 0–20 cm soil depth, while the other three orchards did not vary significantly between these two methods. Moreover, chloroform labile C and MBC generated by CFE-ST and SIR, respectively, are comparable (as they are statistically at par) with each other in surface soil, except plum and peach orchards. Therefore, in surface soil, it is assumed that these two methods could be deployed interchangeably for measuring chloroform labile C; moreover, biomass C in the selected orchard soils. However, significant difference in the MBC estimates by these two methods does not allow their simultaneous use in subsurface soil. In subsurface soil, this was also true between the CFE-V and SIR methods.

On other side, the SIR method showed significantly lower value of MBC than that measured by the other two methods in subsurface soil. As discussed earlier, this observation also suggests that the proportionality factor (40.04) used to convert the maximum initial respiratory response to MBC was not same for both the depths. Fruit crops and different management practices could have affected the community structure of the microbial populations between surface and subsurface soil in these orchards, and consequently, the appropriate conversion values need to be used for converting the data obtained by SIR into MBC. Such observation could also be due to inefficient substrate uptake. Another reason is extended to the over-dependency on the glucose-utilizing microorganisms. The physiological reaction following glucose addition could have decreased per unit biomass (non-glucose utilizing), and thus overall microbial respiration. Headspace sampling of CO_2 by gas chromatography has also the disadvantage that CO_2 accumulates during the course of incubation and, more importantly, that CO_2 could be absorbed in the soil solution as HCO_3^- in high pH soils (>7.0), which might result in underestimation of MBC (Bailey, Bolton, and Smith 2008). In addition, MBC estimation by the SIR method mainly depends on the proportion of live soil microbial biomass, while labile microbial fractions from the lysed cells of the microbial biomass as well as non-biomass soil organic C are rendered extractable by K_2SO_4 in CFE methods, as a mass parameter. Further, the SIR method detects predominantly bacterial biomass (Ross 1991). It is obvious that the proportion of soil microbial biomass is comparatively less in subsurface compared to surface soil due to low substrate availability. Storage of samples at 4 °C over a certain period could lead to the mortality of some microbial biomass, which could decrease the proportion of live microbial biomass in samples. This could probably be the most important reason the SIR method has generated the lowest means of MBC estimates among the three methods in subsurface soil.

The coefficient of variation (CV) in the C extracts from the CFE-V and CFE-ST methods ranged between 1.3% and 28.9%, and 7.1% and 19.5% in surface soil, respectively (Figure 1a, b). The same in subsurface soil varied from 2.1% to 20.6%, and 1.0% to 20.5%, respectively. The SIR method has resulted in CV that ranged from 4.2% to 33.5%, and 5.1% to 22.9% in surface and subsurface soil, respectively (Figure 1c). The depth-wise CV varied between 13.8% and 13.0% for the CFE-ST method and between 14.1% and 9.4% for the CFE-V method, and 13.7% and 12.9% for the SIR method in surface and subsurface soil, respectively (Figure 1d). In surface soil, little variation in the depth-wise CV generated by

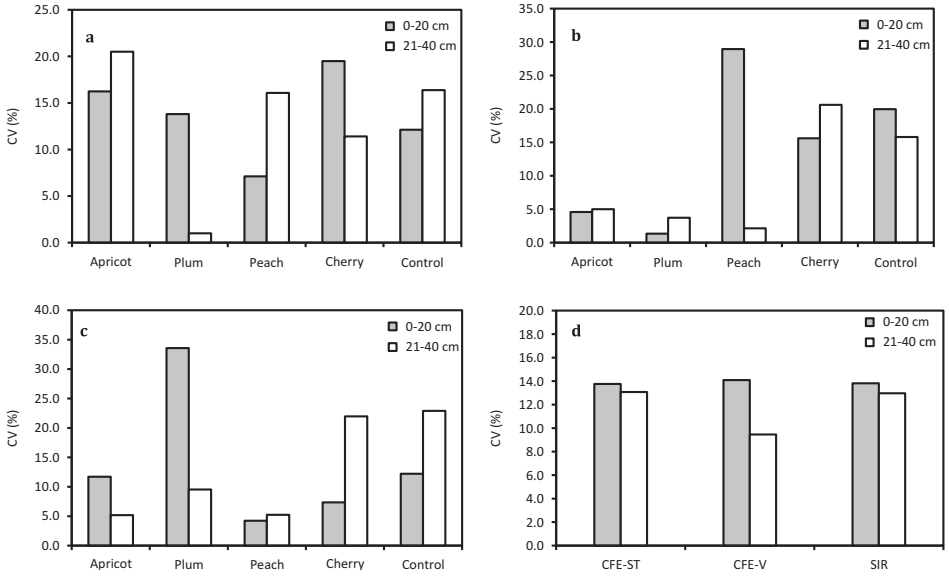

Figure 1. Coefficient of variation (CV) of chloroform labile C measured by (a) Snyder–Trofymow method (CFE-ST) and (b) Vance method (CFE-V); biomass C by (c) substrate-induced respiration method (SIR); and (d) depth-wise coefficient of variation among the three methods.

different methods has indicated that methods are perhaps identical, at least in statistical sense. Similar observation was also made between the CFE-ST and SIR methods in subsurface soil. The CV observed in this study for the CFE-V and SIR methods lies well within the range as reported by many authors (Bailey, Bolton, and Smith 2008; Setia, Verma, and Marschner 2012; Wardle and Ghani 1995), and hence, these methods are perhaps comparable with each other. Nevertheless, CV recorded from the SIR method probably makes it more precise than the other two methods, particularly in surface soil. In other words, the lower the variability in CV (by calculating the difference between the highest and lowest CV within a particular method), the higher is the precision of the values obtained under a particular method at a specified soil depth. It is worth mentioning that when averaged over all the plantations the SIR method showed lowest CV (13.71%) than the other methods in 0–20 cm, while the CFE-V method showed lowest CV (9.45%) in 21–40 cm soil depth. Therefore, in terms of CV in data, the SIR method had a little edge over the other two methods.

The multiple linear regression analysis showed that the relationship of chloroform labile C from the two methods and MBC estimate by the SIR method was significantly affected by soil organic C and available major nutrient levels (Table 3). In addition, it showed very close agreement between the methods (CFE) and soil properties like organic C and available major nutrients, which suggest that both methods could be used for assessing chloroform labile C, which in turn suggest MBC in these orchard soils. However, differences do exist toward the contribution of these soil properties in the variability of chloroform labile C estimates by the two CFE methods. More than 90% (91.7–99.0%) of the variations by different methods could be explained by the independent variables considered in 21–40 cm soil depth, whereas the influence of the independent variables in the variability of chloroform labile C estimates was much narrower (66.8–81.3%) in 0–20 cm soil depth. Crop management practices generally influence the soil chemical properties, particularly in upper soil layer (up to 15 cm), which in turn indicates the microbial activity. However, in deeper soil layer the influence of those management practices diminishes and root exudates and residues play a vital role in governing the soil properties. Thus, it could be expected that microbial biomass would be more dependent on the soil properties in subsurface soil regulated by the root activity of these temperate fruit crops. The higher R^2 value recorded in subsurface soil over surface soil could explain such observation. The soil chemical properties served as

Table 3. Multiple linear regression analysis of additional influence of some selected soil properties on chloroform labile C estimates by CFE-V and CFE-ST methods, and biomass C by SIR method at two soil depths.

	Coefficient	*t*-Value	R^2 (%)	Coefficient	*t*-Value	R^2 (%)
Independent variable	Surface soil (0–20 cm)			Subsurface soil (21–40 cm)		
Model fitting results for CFE-ST						
Constant	2524.1	1.01*	74.2	-3769.8	-1.12*	93.6
pH	-273.1	-0.75**		700.8	1.63**	
Organic C	12.8	0.03**		265.4	0.72***	
Moisture	4.17	0.96		-51.1	-1.70**	
Available N	-4.82	-1.01*		-2.1	-0.53****	
Available P	0.04	0.01**		-6.4	-0.73**	
Model fitting results for CFE-V						
Constant	-601.8	-0.13	66.8	-10.3	-0.01**	91.7
pH	383.3	-0.55**		137.0	0.53	
Organic C	328.0	0.55**		225.4	1.02**	
Available N	-1.5	-0.24		-1.3	-0.57*	
Available P	-16.8	1.18**		0.2	0.03**	
Model fitting results for SIR						
Constant	-913.2	-0.38**	81.3	701.3	0.49*	99.0
Moisture	29.6	0.70***		-15.5	-1.06***	
Organic C	-143.4	-0.37****		193.2	1.08****	
Available N	-5.2	-1.14*		0.1	0.03****	
Available P	-6.5	-0.61**		-1.9	-0.46***	
Available K	0.90	0.44***		0.4	0.54**	

CFE-V, chloroform labile C measured with Vance method; CFE-ST, chloroform labile C measured with Snyder–Trofymow method; SIR, biomass C measured with substrate-induced respiration method.
* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

better predictors (based on R^2 values) of microbial biomass C estimated by the SIR method. This observation makes the SIR method more suitable for measuring MBC in the selected orchard soils. The correlations between CFE-V and CFE-ST (r = 0.80), between CFE-ST and SIR (r = 0.76), and between CFE-V and SIR (r = 0.86) were found to be significant (Table 4). Correlation study indicated the existence of strong relationships between chloroform labile C (including the SIR method) with soil organic C and available major nutrients. It also indicates that there is no consistent evidence of any pair of methods significantly (at least in a statistical sense) more strongly related to each other than any other pair of methods, meaning that they appear to perform roughly equally as predictors of one another. This observation is further strengthened by the fact that the magnitude of relationship between chloroform labile organic C estimation methods and soil chemical properties is somewhat similar, meaning that they followed similar kinds of trend. Nevertheless, amongst the methods, SIR exhibited the strongest relationship with the soil chemical properties.

Conclusions

The methodology of estimation of MBC should ideally be sensitive enough to separate out the differences, if any, among the treatments; here it comprises various fruit crops under temperate climate. The very close agreement between the methods suggests that over this narrower range (i.e., smaller geographical area) all methods are appropriate for assessing MBC. Nevertheless, SIR could, preferably, be recommended for estimation of MBC in soils under temperate fruit crops due to its greater sensitivity and relationships with soil chemical properties (based on R^2 value), rapidity, and ease of measurement. The results of this study have indicated that CFE-ST could be used as an alternative to the CFE-V method for estimating chloroform labile organic C in K_2SO_4 extracts. However, the use of this method would be more worthy especially when large the numbers of samples are needed to be processed within a short period. We would, therefore, suggest the scientific community working in this field to develop a specific relationship between CFE-ST and other methods of chloroform labile organic C estimation, which would allow us to use this method to soils with wide range of properties.

Table 4. Pearson's correlation coefficient (*r* values) of variables related to various soil properties.

	A	B	C	D	E	F	G	H	I
A	1.00								
B	0.80**	1.00							
C	0.86**	0.76**x	1.00						
D	−0.32	−0.11	−0.47**	1.00					
E	0.77**	0.71**	0.84**	−0.29	1.00				
F	−0.01	−0.02	−0.12	0.25	−0.06	1.00			
G	0.73**	0.68**	0.79**	−0.47**	0.77**	−0.23	1.00		
H	0.51**	0.33	0.46**	−0.40*	0.42*	−0.05	0.57**	1.00	
I	0.60**	0.69**	0.74**	−0.46**	0.57**	−0.14	0.71**	0.33	1.00

A, chloroform labile C measured with Vance method; *B*, chloroform labile C measured with Snyder–Trofymow method; *C*, biomass C measured with substrate-induced respiration method; *D*, soil pH; *E*, organic C; *F*, soil moisture; *G*, available N; *H*, available P; *I*, available K.

* $p < 0.05$; ** $p < 0.01$.

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