

Short communication

Limitations of soil microbial biomass carbon as an indicator of soil pollution in the field

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Abstract

The size of the soil microbial biomass carbon (SMBC) has been proposed as a sensitive indicator for measuring the adverse effects of contaminants on the soil microbial community. In this study of Australian agricultural systems, we demonstrated that field variability of SMBC measured using the fumigation–extraction procedure limited its use as a robust ecotoxicological endpoint. The SMBC varied up to 4-fold across control samples collected from a single field site, due to small-scale spatial heterogeneity in the soil physicochemical environment. Power analysis revealed that large numbers of replicates (3–93) were required to identify 20% or 50% decreases in the size of the SMBC of contaminated soil samples relative to their uncontaminated control samples at the 0.05% level of statistical significance. We question the value of the routine measurement of SMBC as an ecotoxicological endpoint at the field scale, and suggest more robust and predictive microbiological indicators.

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The term soil microbial biomass (SMB), i.e. the living part of soil organic matter, is used to describe the total mass of microorganisms present in a soil (Brookes, 1995). The importance of the SMB in soil functioning is well recognised (Dalal, 1998; Stockdale and Brookes, 2006) and SMB has long been suggested to be a significantly more sensitive indicator of changing soil conditions than the total soil organic matter content (Powlson and Jenkinson, 1976). In ecotoxicological studies, the SMB has been proposed as a sensitive endpoint to define the impact of contaminants such as metals on soil biological functioning (Brookes and McGrath, 1984; Brookes, 1995; Dahlin et al., 1997; Giller et al., 1998). The size of the SMB pool is

routinely measured and expressed as carbon contained in the SMB (SMBC), and less frequently as nitrogen contained in SMB. Recently, Stockdale and Brookes (2006) reviewed in detail the methodological evolution of SMBC determination, developments in studying microbial dynamics over the last century, and how these advances have contributed to our understanding of crucial soil processes such as nutrient and carbon cycling.

While we acknowledge the value of quantifying the size of the SMBC *per se*, we do question the value of routinely measuring SMBC to assess potential contaminant effects on the biological functioning of the soil system in field-based trials. In such studies, a decrease in SMBC relative to a control soil is taken to indicate an adverse effect of a contaminant. However, the high spatial and temporal variability in SMBC commonly observed at a field scale leads one to question the value of the SMBC measurements, where the number of replicate soil samples

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examined is low. Unlike controlled laboratory studies, which routinely use homogenised soils, field soils provide a highly variable physiochemical environment. Field topology leads to local differences in water content and temperature, both of which are primary drivers of SMBC, while variability in root distribution, crop residues and presence of macropores are also major sources of variability. This level of variation is often inadequately considered, resulting in data that are unlikely to detect any significant difference in the size of the SMBC pool, and does little to improve our understanding of the effects of contamination on the microbiological functioning of the soil system.

With a view to demonstrate the limitations associated with using SMBC as an indicator of the potential contaminant effects, we investigated the variability of SMBC determined from the commonly used fumigation–extraction procedure (Vance et al., 1987; Wu et al., 1990), with a 10-d fumigation period. Soil samples were obtained from 6 field trials established as part of the Australian National Biosolids Research Program (see Broos et al., 2007; McLaughlin et al., 2006) to investigate effects of metals (Cd, Cu and Zn) on agricultural land. Two weeks following metal salt application, 4 top soil samples (0–10 cm) were collected from each plot using a 5-cm diameter auger. Soil samples were air dried at 40 °C, sieved (<2 mm) and stored in air-tight containers at room temperature prior to chemical and microbiological analysis. Under normal environmental conditions Australian topsoils often attain water contents equal to or lower than the air-dried condition established by drying at 40 °C. It is unlikely that the air drying process used in this study would perturb the microbial population beyond natural conditions. Furthermore, prior to SMBC determination, soils were brought to 50% maximum water holding capacity and preincubated for 14 d in darkness to minimise effects of sampling time, soil temperature and moisture status of field soils at time of sampling. All samples were analysed in duplicate.

Power analysis was used to ascertain the number of independent replicates required to determine a significant reduction ($x\%$) in the size of the SMBC pool, as a function of the variation measured in control soils ($p = 0.05$ and power = 0.8). The sample size calculation assumed normal distribution, equal variance and equal sample size for the two groups (i.e. control and contaminated soil groups). As the variation of the control group is likely to be larger than the contaminated group, the calculated sample size was conservative. The larger the variation associated with the control plots, and the smaller the desired detection difference ($x\%$), the greater the number of field replicates required. We determined the number of field replicates required to claim that a 20% and 50% decrease in the size of the SMBC pool was statistically significant at $p = 0.05$ by using the data from the control plots only (no metal addition).

The average size of the SMBC pool across field trials varied from 216 to 557 $\mu\text{g C/g}$ dry soil (Table 1). Within field trials, the SMBC of uncontaminated soils varied up to 4-fold (e.g. 162–659 $\mu\text{g C/g}$ dry soil at the Avon site) leading to large standard deviations around the average SMBC of control plots (Table 1) which equates to coefficients of variation between 10% and 48%. The number of field replicates necessary to claim that a 50% decrease in the size of the SMBC pool was significant (at $p = 0.05$), varied between 3 and 16 (Table 1, average of 7 across all sites). However, a 50% decrease in SMBC is a drastic effect and typically decreases of the order of 20% or even 10% are used in ecotoxicological studies to derive soil guidelines for contaminants. When it was desired to claim that a 20% decrease in SMBC was significant at $p = 0.05$, the analysis indicated a requirement to collect and measure SMBC on 9–93 field replicates per treatment (Table 1, average of 33 across all sites). We believe that these high replicate numbers required for this endpoint are too high for use in ecotoxicological risk assessment.

The key to developing good indicators of ecotoxicological endpoints requires more robust data measurements

Table 1
The mean and standard error (SE) of the soil microbial biomass carbon (SMBC) for field replicates of control soil samples from 6 Australian field sites and the number of field replicates that would be required to show a decrease in SMBC of 20% and 50% compared to the control soil, using a significance level of $p = 0.05$

Field site	Soil type ^a	pH ^b	OC ^c (%)	Number of control samples collected	SMBC ($\mu\text{g C/g}$ dry soil)		Field replicates required to detect	
					Mean	SE	20% effect	50% effect
Avon	Calcarosol	7.6	1.2	10	436	41	36	7
Dookie	Dermosol	4.9	2.0	6	272	17	10	3
Dutson Downs	Podosol	4.0	5.6	11	234	18	27	6
Flat Paddock	Chromosol	4.4	1.2	5	216	47	93	16
Night Paddock	Chromosol	5.1	3.4	4	461	33	9	3
Tintinara	Sodosol	6.3	1.8	5	557	52	19	4

Soil types and major properties measured as per Broos et al. (2007) are presented.

^aSoil types using the Australian soil classification system (Isbell, 1996).

^bSoil pH measured in 0.01 M CaCl₂.

^cOC = organic carbon.

(Giller et al., 1998; Broos et al., 2005) that can be used in establishing broad-scale effects of contaminants on soil microbial communities. Another issue related to the use of the SMB to establish ecotoxicological guidelines centres on the assumption that the SMB is a single undifferentiated material with a single susceptibility to soil contaminants. The size of the SMB pool does not necessarily yield any information about process rates or microbial diversity and resilience. Moreover, recent developments demonstrate that specific microbial communities can become more tolerant to contamination without compromising function (Bååth et al., 1998; Fait et al., 2006; Mertens et al., 2006). With improved analytical methods, studies of single soil species (e.g. rhizobia: Lakzian et al., 2007) or soil microbial functions (e.g. soil nitrification: Smolders et al., 2001) are becoming increasingly powerful, and have considerable potential in providing more meaningful information which can be interpreted in unravelling process level effects of contaminants. Though the SMB is still ‘the eye of the needle’ through which all organic matter must pass as it is mineralised to its basic inorganic components (Jenkinson, 1977), we believe a focus on measurement of the rates at which components of soil organic matter pass through the eye, rather than the measurement of the size of the eye itself, is needed in soil microbial ecotoxicology.

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