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RESEARCH ARTICLE

Correlation between carbon dioxide evolution and biological quality index of long-term nutrient management adopted soils

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ABSTRACT

Soil respiration in terms of carbon dioxide (CO_2) evolution is a potential biological indicator to assess the soil quality as influenced by different agricultural practices. However, the relation between the CO_2 evolution and the biological attributes of soil is a multitude and hence less resolved. The purpose of the study is to relate the measured CO_2 values with the biological quality indicators of the soil. Four long-term nutrient management treatments (organic manure amended, OM, integrated nutrient management enforced, INM, synthetic fertilizer applied, IC, and unfertilized control, Control) from 100-years old permanent manurial experiment was used for this study. The soils were analyzed for soil organic carbon, microbial biomass carbon, labile carbon, protein index, dehydrogenase activity, and substrate-induced respiration and calculated the soil biological quality index (SBQI) by quadrant-plot method. All the soils were measured for the flush of CO_2 immediately after wetting the dry soil by three methods viz., alkali trap method, infra-red probe-based flux apparatus, and multi-gas analyzer. The results revealed that INM had a positive influence to improve all the six biological attributes followed by OM, while the IC and Control had at par levels of all the assessed variables. The SBQI also has high resolution to discriminate against the soils and INM > OM > IC ≈ Control is the order being observed. The CO_2 evolved from these four soils had a strong positive correlation with all the assessed biological health indicators. The regression analysis revealed that SBQI had a significant positive relation with alkali trap CO_2 (R^2 0.82), flux CO_2 (R^2 0.75), and multi-gas analyzer CO_2 (R^2 0.79). The present results suggest that analyzing CO_2 evolution could be a simple method to relate the overall biological quality of the soil. Hence, developing a simple tool to measure the soil CO_2 evolution could help the farmers to know the biological quality of the soil .

Keywords: *Soil health; Soil quality index; Biological indicators; Sustainable soil management.*

INTRODUCTION

Soil quality or health is the ability of a soil to function within the ecosystem to sustain productivity, maintain environmental quality, and promote plant and animal health (Doran and Parkin, 1994). Monitoring soil quality is an important component of any land management system that sustains soil resources (Morrow *et al.*, 2016). Soil quality explores the physicochemical, biochemical, and biological attributes of the soil to determine whether the soil function is enhancing, maintaining, or deteriorating due to different land use patterns and agricultural practices (Bünemann *et al.*, 2018). A common universal method to determine the soil quality is unavailable, as the soil system functioning is controlled by the soil types, agro-ecosystems, and soil management practices (Rinot *et al.*, 2019). Hence, soil quality indicators, a minimum set of soil attributes, are used to assess the soil quality. The criteria for a good indicator should be vital for the soil functioning, informative, sensitive to management practices, well-defined and standardized procedure with simple and cost-effective and easy to interpret with well-defined ranges and trends (Apfelbaum *et al.*, 2019). Total organic carbon, bioavailable nitrogen, soil bulk density, macro-aggregates stability, microbial biomass carbon, labile or particulate or soluble carbon are some of the potential quality indicators used to monitor the soil health as influenced by nutrient management, tillage, cropping pattern and other activities

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(Andrews *et al.*, 2004; Karlen *et al.*, 2006; Masto *et al.*, 2008; Bai *et al.*, 2018).

Soil quality index (SQI) is the minimum set of parameters that, when interrelated, provides numerical data on the capacity of soil to carry out one or more functions (Acton and Padbury, 1993). SQI is complex due to diverse soil quality indicators and their difference in range and response to the managements and difficult to integrate them as a single measurable unit (Garcia *et al.*, 1994). Yet, several attempts were made to develop SBI using a different set of quality indicators for a specific soil type. Simple addition or scoring function of soil quality indicators are two approaches widely followed for SQI calculations (Mukherjee and Lal, 2014; Moebius-Clune *et al.*, 2016). However, SQI procedures give more weightage to the physicochemical indicators such as soil aggregation, moisture-holding capacity, carbon dynamics, nutrient carrying capability, and the biological attributes, the key players of soil quality, get less attention (Biswas *et al.*, 2017; Pulido *et al.*, 2017; Calero *et al.*, 2018; Menta *et al.*, 2018; Schmidt *et al.*, 2018). Parisi (2001) developed the 'Soil biological quality index' (SBQI) to emphasize biological attributes to the soil quality. Pascazio *et al.* (2018) used microbial biomass, β glucosidase, mineralizable nitrogen, and urease to measure the SBQI. Similarly, Vincent *et al.* (2018) used bacterial and fungal density and richness with mycorrhizal colonization as SBQI indicators. Aravindh *et al.* (2020) 10 scaled SBQI using soil organic carbon, microbial biomass carbon, soil labile carbon, soil protein index, dehydrogenase, and substrate-induced respiration and assessed it for long-term nutrient management adopted soils.

Soil tests are generally used to recommend the additional nutrients required for a crop based on the plant-available nutrient status of the soil. In conventional chemical testing procedures, apart from macro- and micronutrients, soil organic carbon is quantified as a representative of biological property. However, the present soil testing methods focus the soil's capacity to supply nutrients to a crop and they were not assigned to measure the biological processes to monitor the soil quality. Any soil assessment that can improve our knowledge to predict crop growth and soil health for agricultural sustainability would be welcome progress (Bavougian *et al.*, 2019). Hence, the concept of soil quality rather than soil fertility is much interest in recent years. Cornell University, USA developed comprehensive soil health assessment (Moebius-Clune *et al.*, 2016) and Soil quality institute, USDA developed Soil Assessment and Management Framework (Wienhold *et al.*, 2009) integrating physical, chemical and biological attributes of the soil. These two approaches are robust with many detailed procedures and need well-equipped soil testing laboratories for assessment. Alternatively, simple and direct monitoring soil quality has recently generated much interest among the farmers to measure and monitor their efforts to improve the soil properties that associate with soil health. Solvita® and Haney soil health test® are such kits developed for the USA farmers to assess by themselves. Solvita test® quantifies the amount of carbon dioxide (CO_2) that evolved over 24-h after rewetting the soil samples and correlates with microbial activity, mineralizable nitrogen, and phosphorus (Haney *et al.*, 2008). 2008). In Haney test®, organic acids were used as extractants to accurately measure the plant available nutrients (Haney *et al.*, 2010). However, no attempts were made in India to develop a farmers' friendly tool to monitor the soil's biological properties to sustain the soil quality. The recent works strongly correlate the CO_2 evolution following rewetting of dried soil (referred to as flush of CO_2) with mineralizable nitrogen and phosphorus of soil. Several studies used the flush of CO_2 to characterize soil biology as influenced by tillage, crop management, crop sequence (Morrow *et al.*, 2016; Franzluebbers, 2018; Franzluebbers and Haney, 2018; Franzluebbers *et al.*, 2018; Franzluebbers, 2020; Franzluebbers and Veum, 2020). In the present work, we assume that the CO_2 evolved from the soil samples may have a positive and strong correlation with soil biological attributes and by measuring the CO_2, we can presumably derive the biological quality of the soil. For this, we measured the biological attributes of the soils from 100-years old permanent manurial experiment, calculated the soil biological quality index, and related with the flush of CO_2.

MATERIAL AND METHODS

Experimental site and soil sampling

The long-term permanent manurial trial being maintained by the Department of Soil Science and Agricultural Chemistry, Tamil Nadu Agricultural University, Coimbatore, India was selected for this investigation. The details of the study area, trial details, and their basic soil characteristics were described in Table 1. We have selected four continuously treated long-term nutrient management-adopted soils for our investigation *viz*., absolute control soil (control); inorganic fertilizers applied to soil (IC); organic amendment applied to soil (OM) and integrated nutrient management (both organic and inorganic) adopted soil (INM). The details of each treatment are as follows: Control represents the plot in which the crop (maize followed by sunflower) was raised without any nutrient amendments. The soils with leftover crop residues were incorporated during tillage. In IC, nitrogen (N), phosphorus (P) and potassium (K) were applied

in the form of urea, superphosphate, and more of potash at recommended dosage specific to the crops (maize – 250:75:75 kg NPK/ha; sunflower – 60:90:60 kg NPK/ha. OM plot was applied with farmyard manure alone as a nutrient amendment (12.5 t/ha of farmyard manure, FYM, irrespective of the crop). The well-decomposed manure was incorporated into the soil during the last plowing before sowing every crop. INM refers to the plot with 100% NPK as chemical fertilizers along with FYM (12.5 t/ha) (similar to IC and OM, respectively). All the plots were plowed using country-plough, added with different nutrient amendments, and leveled manually and the crops were raised as per the standard practice.

To avoid the crop influences on the assessed soil variables, soil samples were collected from the upper 15 cm of the surface soil of each plot during the fallow period, when the crop was not raised (July 2019). In each plot, ten sub-sample soil cores were collected randomly and pooled together in a composite sample and likewise, four replicates per soil were taken. The debris, plant residues, and stones were removed during sampling to avoid any influence on soil parameters analyzed. The soil samples were packed in plastic bags, transported to the laboratory using an ice cooler box, and stored at -20°C. The gravimetric moisture content of the soil was measured immediately.

Soil biological properties

Soil organic carbon (SOC) was analyzed by wet chromic acid digestion method (Walkley and Black, 1934) and expressed as mg per g of soil. The microbial biomass carbon (MBC) was measured by the fumigation-incubation technique (Jenkinson and Powlson, 1976) and expressed as µg per g of soil. Soil labile carbon (SLC) was measured by the permanganate method (Blair and Crocker, 2000) and expressed as µg per g of soil. Soil protein was extracted from soil using a protocol as described by Hurisso *et al.* (2018) and expressed as µg per g of soil. The dehydrogenase (DHA) was measured by the procedure described by (Casida Jr *et al.*, 1964) and expressed as µg of triphenyl formazan released per g soil per day. The substrate-induced respiration (SIR) was measured the rate of respiration in the soil after glucose was amended in it and expressed as µg of CO_2 released/g soil/h (Enwall *et al.*, 2007).

Soil biological quality index

A unitless soil biological quality index (SBQI) was calculated using the data of soil biological attributes by the quadrant-plot method developed by Aravindh *et al.* (2020). In brief, six significantly correlated ($p<0.001$) variable pairs (SOC/MBC, SOC/SLC, SOC/SIR, MBC/SPI, MBC/DHA, MBC/SIR) and their regression coefficient (R^2 values), and mean values were used for the scoring. The paired variables were plotted in a scatter plot using variable-1 (major contributor) in the x-axis and variable-2 (second contributor) in the y-axis. The scatter plot was converted into four quadrants by scaling the mean values of the corresponding variables in their axes. The right-handed upper quadrant representing 'high' for both variables are scaled to 4, as both the variables above the means. The right-handed lower quadrant representing 'high for variable-a and low for variable-b' is scaled to 3. Likewise, the left-handed upper quadrant scored for 2 and the left-handed lower quadrant which represents 'low' for both the variables had the value of 1. Since, the major contributor is always in the x-axis, high for variable-a and low for variable-b had the score value of 3 and its opposite had 2. All the six-pairs were scored using this method and SBQI was calculated as follows:

$$SBQI = \sum(\text{Paired variable score x regression coefficient})$$

Soil carbon dioxide evolution

All the four soil samples were analyzed for carbon-di-oxide evolution by three different methods under laboratory condition.

Alkali trap method: For this, 100 g of soil amended with 15-ml of 1% of glucose and 35 ml of deionized water was placed in an air-tight bottle with a vial of 10 ml of 0.1N NaOH. The bottles were incubated at 30°C for 12 h. The unreacted alkali in the trap was quantified by back-titration with 0.1N HCl to determine the amount of CO_2 evolved from the soil (Franzluebbers and Veum, 2020).

CO_2-flux apparatus method: The same experimental set up was detected for CO_2 levels using the CO_2-flux apparatus (Lutron GC- 2028 digital CO_2 meter with non-dispersive Infra-red sensor) and expressed as mg of CO_2 evolved per 100 g of soil after 12 h of incubation.

Multi-gas analyzer: The same experimental set up was fed to the probe-based multi-gas analyzer (Geotech) and recorded the amount of CO_2 (ppm) evolved after 12 h of incubation.

Statistical analysis

All the data were subjected to analysis of variance and Tukey's test at a 5% significance level using XLSTAT (version 2010.5.05) adding with Microsoft Excel for Windows 2007 to reveal the significant statistical difference among the treatments. The relation between soil variables influenced by long-term nutrient management adoptions was evaluated by Pearson correlation analysis (Pearson, 1895). Simple linear regression was calculated between three different CO_2 evolution methods and the SBQI,

representing all the six biological attributes of soil (Freedman, 2009). Principal component analysis (PCA) was performed for the observed variables of soil to reveal the similarities and differences between samples and to evaluate the relationship between soil samples and observed variables (Wold *et al.*, 1987).

RESULTS AND DISCUSSION

In the present work, we have developed a correlation between the amounts of CO_2 evolved after rewetting with a readily available carbon source (glucose) and the biological attributes of the soil. This will help to provide quick and early information about soil health. First, we have used soil organic carbon, soil microbial biomass, soil labile carbon, soil protein index, dehydrogenase activity, and substrate-induced respiration to scale the SBQI.

Table 1. Study area and soil characteristics

Details	PME, TNAU, Coimbatore
Centre	Tamil Nadu, Coimbatore
Geographical coordinates	11 N, 77 E
Altitude	426 m
Max and Min temperature	34.2°C and 20°C
Annual rainfall	670 mm
Climate type	semi-arid sub-tropical
Year of establishment	1909
Test crop	Maize – Sunflower
Cropping method	Irrigated
Variables	Nutrient management
Soil texture	sandy loam
Soil classification	Typic Haplustalfs
Initial soil characteristics	
pH	8.30
Electrical conductivity (dS/m)	0.25
Soil organic carbon (mg/g)	1.80
Available N (mg/kg)	147
Available P (mg/kg)	4.8
Available K (mg/kg)	381

Soil biological attributes and Soil biological quality index as influenced by long-term nutrient managements

The long-term adoption of integrated nutrient management had significantly improved the SOC (10.5 mg/g) followed by an organic manure amendment (8.34 mg/g).

The synthetic chemical fertilizer applied and unfertilized control plots had at par and the lowest level of SOC (Table 2). However, the INM and OM had at par and the highest quantity of MBC, while IC and Control recorded the lowest levels. All the four soils had 840-860 µg/g of SLC and among the four nutrient managements, INM recorded the highest SLC followed by OM, whereas IC and Control had the lowest level of SLC. All four long-term nutrient management had a significant impact on the soil protein index of the soil. INM > OM > IC > Control is the trend observed in the SPI concentration of the soil. Likewise, the soil dehydrogenase activity and substrate-induced respiration rate also had the same level of significance in four soils (INM > OM > IC > Control) (Table 2).

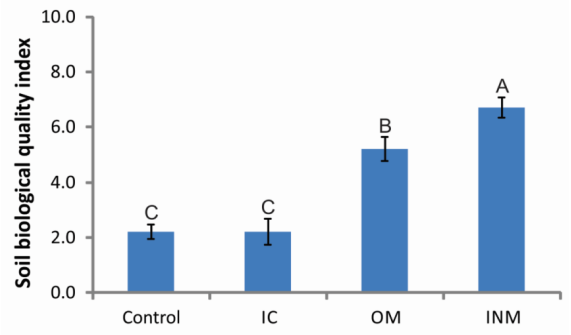

Figure 1. Soil biological quality index calculated based on six biological attributes using the quadrant-plot method in long-term nutrient management adopted soils.

Data represent mean (n=4) and error bars indicate the standard error. For each panel, the different letter indicates significantly different within the treatments at P<0.05 according to Tukey's test. Control - Unfertilized control soil; IC - Inorganic chemical fertilized soil; OM - Organically managed soil; INM - Integrated nutrient management enforced soil.

These two variables resolve the treatment difference better than other parameters. The quadrant-plot based SBQI calculated from the data (SOC, MBC, SLC, SPI, DHA, SIR) using relativeness of the assessed variables (regression coefficient of paired variables) significantly discriminated against the soil samples. The six paired variables were plotted in a scatter plot and the mean of both the variables was used to form quadrants of the plot.

Table 2. Impact of long-term nutrient management adoptions on biological attributes of soil

Treatments	SOC (mg/g)	MBC (µg/g)	SLC (µg/g)	SPI (µg/g)	DHA (µg/g)	SIR (µg/g)
Control	5.40 (± 0.21)^{C}	838.02 (±30.98)^{B}	848.72 (± 1.58)^{C}	117.38 (± 0.52)^{D}	0.54 (± 0.01)^{D}	1.78 (± 0.06)^{D}
IC	6.12 (± 0.07)^{C}	832.87 (±30.66)^{B}	846.28 (± 0.41)^{C}	131.42 (± 1.41)^{C}	0.60 (± 0.01)^{C}	2.40 (± 0.03)^{C}
OM	8.34 (± 0.26)^{B}	1171.13 (±30.93)^{A}	854.42 (± 1.36)^{B}	138.27 (± 0.43)^{B}	0.78 (± 0.02)^{B}	2.97 (± 0.02)^{A}
INM	10.50 (± 0.35)^{A}	1127.34 (±30.92)^{A}	862.94 (± 0.74)^{A}	159.45 (± 1.13)^{A}	0.88 (± 0.01)^{A}	2.64 (± 0.04)^{B}

Data represent mean (± SE) (n=4) and in each column, values followed by different letter indicates significantly different at P < 0.05 according to Tukey's test. Control - Unfertilized control soil; IC - Inorganic chemical fertilized soil; OM - Organically managed soil; INM - Integrated nutrient management enforced soil. SOC – Soil organic carbon; MBC – Microbial biomass carbon; SLC – Soil labile carbon; SPI – Soil protein index; DHA – Dehydrogenase; SIR – Substrate induced respiration; SBQI – Soil biological quality index.

The samples positioned in the quadrants were scored (scaled from 1 to 4) and the score values were weighed with the regression coefficient (R^2) and scaled to 10. Among four soil samples, INM had SBQI of 6.42, while OM - 5.20, IC - 2.22, and Control – 2.21 (Figure 1). INM > OM > IC ≈ Control was the trend observed in the soil samples.

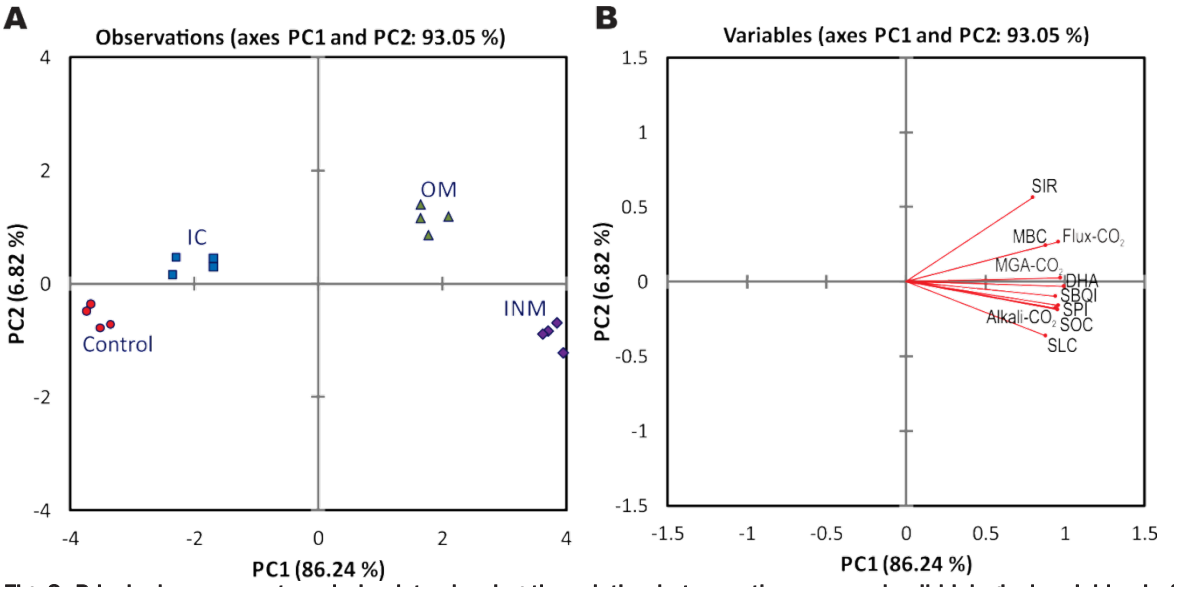

Figure 2. Principal component analysis plots showing the relation between the assessed soil biological variables in four different nutrient management adopted soils.

(A) Scoring plot showing the positions of the soils; (B) Loading plot showing orthogonal positions of assessed soil variables. The % variance explained by each component (PC1 and PC2) is given in parentheses in axes. SOC - Soil organic carbon; MBC - Microbial biomass carbon; SLC - Soil labile carbon; SPI - Soil protein index; DHA - Dehydrogenase activity; SIR- Substrate-induced respiration; SBQI – Soil biological quality index. Control - Unfertilized control soil; IC - Inorganic chemical fertilized soil; OM - Organically managed soil; INM - Integrated nutrient management enforced soil.

These six soil attributes and their role are well-known in soil functioning. Apart from that, these variables are known for consistent performance as indicators, relatively quick, and simple assessment and sensitive to soil disturbances. We measured these six variables from four long-term nutrient management adopted soils (control, inorganic fertilizer-applied, organic manure amended, and integrated nutrient management adopted). Based on the literature and our previous works, the continuous use of organic and inorganic nutrient amendments could significantly alter the above mentioned biological attributes and largely SOC (Preethi *et al.*, 2012; Chinnadurai *et al.*, 2013; Balachandar *et al.*, 2014; Chinnadurai *et al.*, 2014; Tamilselvi *et al.*, 2015; Balachandar *et al.*, 2016).

Table 3. Impact of long-term nutrient management adoptions on soil carbon dioxide levels measured through different methods

Treatments	Alkali trap-CO_2 (mg/100 g soil)	Flux-CO_2 (mg/100 g soil)	MGA-CO_2 (mg/100 g soil)
Control	8.25 (± 0.55)^{D}	168.25 (6.26)^{C}	0.32 (0.01)^{D}
IC	11.60 (± 0.54)^{C}	252.00 (4.32)^{B}	0.44 (0.02)^{C}
OM	14.30 (± 0.64)^{B}	460.75 (5.98)^{A}	0.53 (0.01)^{B}
INM	15.40 (± 0.90)^{A}	451.50 (5.04)^{A}	0.64 (0.02)^{A}

Data represent mean (± SE) (n=4) and in each column, values followed by different letter indicates significantly different at P < 0.05 according to Tukey's test. Control - Unfertilized control soil; IC - Inorganic chemical fertilized soil; OM - Organically managed soil; INM - Integrated nutrient management enforced soil.

All these biological attributes were reported highest in INM and OM, whereas the IC and control recorded on par values or sometimes IC was higher than control. Hence, the scale developed using these six variables should discriminate against the OM, INM, IC, and control to each other. The SBQI calculated using the quadrant plot approach could significantly discriminate against the soils based on the assessed quality indicators (Figure 1 of the present work). INM with the highest SBQI followed by OM, and least in IC and Control reported in the present experiment agrees with previous reports (Mukherjee and Lal, 2014; Biswas *et al.*, 2017; Schmidt *et al.*, 2018; Aravindh *et al.*, 2020). In this method, SOC and MBC are considered as major drivers of the biological activity, while the rest of the four (SLC, SIR, DHA, SIR) are indicating their function in the soil ecosystem.

Table 4. The correlation coefficient (Pearson (n-1)) relating all the biological attributes with soil CO_2 quantification in long-term nutrient management adopted soils

Variables	SOC	MBC	SLC	SPI	DHA	SIR	SBQI	Alkali-CO2	Flux-CO2	MGA-CO2
SOC	1.00*									
MBC	0.79*	1.00*								
SLC	0.88*	0.74*	1.00*							
SPI	0.93*	0.71*	0.85*	1.00*						
DHA	0.96*	0.87*	0.90*	0.93*	1.00*					
SIR	0.67*	0.76*	0.50NS	0.68*	0.77*	1.00*				
SBQI	0.90*	0.83*	0.86*	0.85*	0.92*	0.68*	1.00*			
Alkali-CO2	0.93*	0.75*	0.87*	0.96*	0.94*	0.67*	0.91*	1.00*		
Flux-CO2	0.89*	0.92*	0.76*	0.84*	0.94*	0.90*	0.87*	0.85*	1.00*	
MGA-CO2	0.93*	0.80*	0.80*	0.94*	0.97*	0.81*	0.89*	0.94*	0.92*	1.00*

* Correlation is significant at the 0.05 level; NS – Non-significant. SOC – Soil organic carbon; MBC – Microbial biomass carbon; SLC – Soil labile carbon; SPI – Soil protein index; DHA – Dehydrogenase; SIR – Substrate induced respiration; SBQI – Soil biological quality index.

Soil CO_2 evolution and its relation with soil biological attributes

All three methods adopted to assess the soil CO_2 evolution under laboratory conditions could discriminate against the soil samples based on long-term nutrient management. The alkali-trap method recorded the soil CO_2 with a range of 8 – 15 mg/100 g soil and among the four soils, and INM had the maximum CO_2 (15.4 mg) followed by OM (14.3 mg) (Table 3). CO_2 flux-apparatus recorded 10-fold higher levels of soil CO_2 (ranged 160-450 mg) than the alkali-trap method. The net CO_2 exchange was higher owing to the fact that soil alone was used without any systems for a net CO_2 uptake and a negative or low CO_2 flux. The OM □INM > IC > Control is the tendency noted in this method as well. The multi-gas analyzer also recorded the same trend of CO_2 released from the soil (INM > OM > IC > Control).

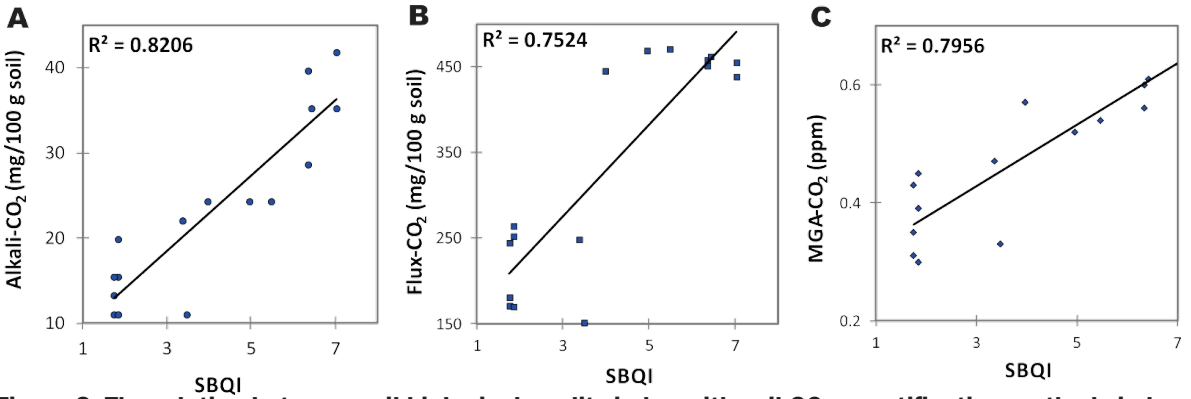

Figure 3. The relation between soil biological quality index with soil CO_2 quantification methods in long-term nutrient management adopted soils.

A - Alkali-trap method; B – Flux apparatus method; C – Multi-gas analyzer method. R2 – regression coefficient (linear).

The observation plot showing the positions of soil samples (INM, OM, IC, Control) and loading plot presenting the orthogonal positions of observed variables of soil (biological attributes, SBQI, CO_2 evolution) explained by the first two components (PC1 and PC2) are presented as Figure 2. The PC1 showed 86.24% variability to the total variability (93.05%), while the PC2 adds 6.82% to it. All the four nutrient management adoptions following for more than 100-years discriminated against the soil samples through the observed variables (Figure 2A). The Control samples were positioned in a negative quadrant (PC1 –ve; PC2 –ve) of the PCA plot, while the IC was positioned in PC1 –ve and PC2 +ve quadrant of the plot. OM is positioned in the upper-right quadrant (PC1 and PC2 +ve), while the INM is positioned in the lower right quadrant (PC1 +ve and PC2 –ve). INM and OM had a positive correlation with the observed variables, which lay in the same orthogonal positions of loading plot, while IC and Control correlated negatively to the soil attributes. The contribution of variables to PC1 and PC2 variability was derived from the PCA to measure the role of each variable to the PCA-based variability. All the ten assessed variables are significant contributors to PC1 and the level of contribution of each variable are DHA - 11.4%, MGA-CO_2 - 10.9%, SOC – 10.7%, flux CO_2 – 10.6%, alkali CO_2 – 10.5%, SBQI – 10.2%, SPI – 10.2%, MBC – 9.0%, SLC – 9.0% and SIR – 7.4%. For PC2, SPI (46%), SLC (19.5%), flux-CO2 (10%) are the significant contributors.

In correlation analysis, all the six biological variables and calculated SBQI were positively correlated with the CO_2 evolution of soil. All the three methods (alkali-CO_2, flux-CO_2, MGA-CO_2) had a significant positive correlation ($p<0.05$) with SOC, MBC, SLC, SPI, DHA, SIR, and SBQI (Table 4). The Pearson correlation coefficient ranged between 0.71-0.96 was recorded between the pairs of variables. Considering SOC as a key attribute to a variably nutrient manages soil on a long time basis, a positive strong correlation was evident with DHA (0.96) and SPI (0.93). This is suggestive of a perfect positive linear relationship between these two variables. SLC and SIR are the only insignificant correlated variable pairs observed in this investigation. The linear regression coefficient (R^2) was calculated with soil CO_2 evolution as the dependent variable and SBQI as the independent variable showed significance (Fig. 3). All three CO_2 evolution methods had positive and significant R^2 ($p<0.001$) with SBQI. Among the three methods tested, alkali-CO_2 had maximum R^2 (0.82) followed by MGA-CO_2 (0.80) and flux-CO_2 (0.75). Likewise, the R^2 values among the CO_2-evolving methods had significant correlations viz., alkali-CO_2 / flux-CO_2 – 0.72; alkali-CO_2/MGA-CO_2 – 0.88 and flux-CO_2/MGA-CO_2 – 0.85 ($p<0.001$).

In the present work, we related the measured SBQI of the soils of long-term nutrient management with the flush of CO_2 under laboratory conditions. The soil respiration rate or flush of CO_2 could be a potential indicator that could strongly relate several biological constituents including biologically active soil C and N fractions, water-soluble C, C and N mineralization and microbial biomass carbon (Morrow *et al.*, 2016; Sciarappa *et al.*, 2017; Franzluebbers, 2018; Franzluebbers *et al.*, 2018; Franzluebbers, 2020; Franzluebbers and Veum, 2020). The relation was strong across the soil types and different management conditions; hence, it can be developed as a simple, rapid, and reliable method for testing biological quality. To validate this concept to Indian agro-ecological conditions, in the present work, we have measured the CO_2 evolution from the soils under the influence of long-term nutrient management and assessed their relativeness to the biological attributes. Since all the four soils had distinct biological attributes as influenced by long-term nutrient management, the CO_2 evolved from these soils could easily be correlated with the biological quality of the soils (Franzluebbers and Veum, 2020). We have amended glucose uniformly in all the soil samples to accelerate the soil respiration at the earliest so that the result could be obtained quickly. All the six biological attributes from four different soils were significantly correlated with the measured CO_2 (Table 4 of the present work) which is in agreement with previous findings (Franzluebbers, 2018). We also showed that all the three methods *viz.,* alkali trap, flux apparatus, and multi-gas analyzer gave the same resolution. The CO_2 flux analysis also behaved similarly and a positive flux was observable as substrate was added to the experimental soils without any systems for significant net CO_2 uptake. Hence, the methods of CO_2 analysis are thus validated. Further, the present results also showed that the SBQI values measured in four long-term nutrient management-enforced soils have a significant strong relation with the flush of CO_2 tested under laboratory conditions. The INM and OM which recorded the highest SBQI values with the highest CO_2 release followed by IC and Control. The present results are in agreement with the previous reports on organic and conventional nutrient management (Leskovar and Othman, 2018).

CONCLUSION

To conclude, the present results indicate that measuring the amount of CO_2 released immediately after wetting the dry soil could be a potential biological indicator that had a positive and strong relationship with most of the biological attributes of the soil. The amount of CO_2 released from the soil and the biological quality index of the soil as influenced by organic and inorganic nutrient management are with positive correlation. Hence, developing a farmer's friendly tool to measure the flush of CO_2, which will provide basic information about the soil quality status, could be a useful approach for long-term soil health sustainability.

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Ethics statement

No specific permits were required for the described field studies because no human or animal subjects were involved in this research.

Consent for publication

All the authors agreed to publish the content.

Competing interests

There was no conflict of interest in the publication of this content

Data availability

All the data of this manuscript are included in the MS. No separate external data source is required.

If anything is required from the MS, certainly, this will be extended by communicating with the corresponding author through corresponding official mail: dbalu@tnau.ac.in-

Author contributions

Research grant	-	DB
Idea conceptualization	-	DB
Experiments	-	GV
Guidance	-	MM, SK, DB
Writing-original draft	-	GV, DB
Writing- reviewing &editing	-	MM, SK, DB

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