Cellulose Decomposition Potential of Soil as Affected by Vegetable Cultivation: A Case Study in Kegalle District, Sri Lanka

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ABSTRACT

The potential of microbial communities for organic C decomposition is a crucial factor determining CO_2 emissions from soil, C storage, and short-term nutrient turnover. We conducted a study to assess cellulose decomposition potential (CDP) of soils as affected by vegetable cultivation. Ten sites cultivated with vegetables and five sites under natural vegetation, distributed in Atulugama and Kanangama Grama Nilaldhari divisions in Kegalle District of Sri Lanka, were selected for the study. The cultivated lands have been managed with organic fertilizers (n=4), synthetic fertilizers (n=3), or a combination of both types of fertilizers (n=3). Soils collected at 0-15 cm depth were used in a laboratory incubation experiment to assess CDP in triplicates for two weeks. The *in situ* CDP was studied by placing litter bags containing two types of cellulose materials, *i.e.* cotton wool or cellulose filter papers, separately for four and six weeks, respectively. They were placed at 5 cm depth in the field in two replicates. Vegetable cultivation had significantly ($p<0.05$) reduced soil organic C content. There was no significant correlation ($p>0.05$) between CDP observed under laboratory and field conditions. The effect of land management on CDP was significant ($p<0.05$) only in laboratory incubation, in which soils collected from the lands cultivated with synthetic fertilizers alone had nine-fold high CDP compared to uncultivated soils. In situ decomposition of added cellulose filter papers and cotton wool after four weeks ranged from 13-100% and 61-65%, respectively. Nearly 44% of sites exhibited values >80% for CDP. In conclusion, vegetable cultivation affected CDP differently depending on the nature of cellulose input and the history of fertilizer management.

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INTRODUCTION

Soil stores about 1,500 Pg of organic C, approximately twice as much C as in the atmosphere (Lal *et al.*, 2003; Scharlemann *et al.*, 2014). The carbon mineralization and decomposition (CMD) process plays a vital role in land-atmosphere C exchange and soil C storage. Further, the CMD affects the cycling of N, P, and S, contributing to short-term nutrient turnover in soil. Therefore, understanding how land management practices affect soil CMD is critical to foresee CO_2 emissions and develop strategies to increase C sequestration for combating global warming.

Organic materials added to soil are shredded by macrofauna into smaller particles and mixed within the soil profile (Sofo *et al.*, 2020; van Gestel *et al.*, 2003). Through C mineralization (CM), organic C in materials is transformed to inorganic C forms, mainly CO_2. However, a fraction of organic C is sequestered in the soil, transforming gradually from labile substances into more stable humic substances contributing to soil organic C storage. Thus, all the organic C is not immediately mineralized. Therefore, the time taken for complete mineralization of an added material may depend on the number of biotic and abiotic factors (Sofo *et al.*, 2020; Bernal *et al.*, 1998; van Gestel *et al.*, 2003; Hossain *et al.*, 2017). Active-C is a major component of labile-C, and it is largely comprised of polysaccharides, proteins, nucleic acids, and carboxyl carbon freely available in the soil and microbial biomass (Rovira and Vallejo, 2002). Labile soil organic C pools are considered as valuable indicators of changes in soil C sequestration and dynamics induced by different soil management practices, such as application of organic amendments and synthetic fertilizers, tillage, and crop rotation (Haynes, 2000). In soil, the CMD is a complex process that involves many organisms from different strata in the food chain (van Gestel *et al.*, 2003; Blagodatskaya *et al.*, 2014). The efficiency of CMD in the soil is therefore predominantly determined by the functionality of microbial communities.

Previous studies suggest that the CM is a sensitive and reliable measure to study soil microbial activity (Bernal *et al.*, 1998; Hossain *et al.*, 2017). Principles of mass balancing, CO_2 emissions, and tracer techniques (with ^{13}C and ^{14}C isotopes) have been used as an integrative approach to estimate the CMD (Fontaine *et al.*, 2004; Strickland *et al.*, 2010; Blagodatskaya *et al.*, 2014). Decomposition of cellulolytic materials has been suggested as an effective and realistic standardized method for studying heterotrophic microbial assemblages in

soil and water (Harrison *et al.*, 1988; van Gestel *et al.*, 2003; Colas *et al.*, 2019). In addition, litter-bag assays are used commonly to assess organic material decomposition (Boulton and Boon, 1991; Kriauciuniene *et al.*, 2012). Here, a known mass an organic material is placed in mesh bags in the field at the desired soil depth, and later the bags are retrieved to determine mass loss over time (Boulton and Boon, 1991).

Maintaining soil organic matter (SOM) quality and quantity is vital for safeguarding long-term soil fertility and sustainability of agricultural lands (Lal *et al.*, 2003). While promoting the use of management practices to improve SOM, it is crucial to increase awareness among farmers on how soil biological activities support its health. Therefore, tedious, highly technical, and time-consuming standard laboratory techniques may not be practical for the educational programs that target farmers. The objective of this study was to assess the activity of cellulolytic microorganisms in soil as affected by vegetable cultivation, employing methods compatible with visualizing data that can be used in training programs targeting farmers. Here, we determined the cellulose decomposition potential (CDP) using an *in-situ* litter bag study and an *ex-situ* soil incubation experiment. Further, we also investigated the relationship of CDP with total and active-C in soil.

METHODOLOGY

Site description and soil analysis

The study was conducted from March to April 2019 in farm fields at Atulugama (Lattitude. 6.99 N and Longitude. 20.26 E) and Kanangama (Lattitude. 7 N and Longitude. 20.27 E) Grama Nilaldhari divisions in Kegalle District, Sri Lanka. The selected region is in the Wet-zone Low country (WL) agro-climatic zone (Punyawardena *et al.*, 2003). Ten sites cultivated with vegetables and five uncultivated sites under natural vegetation located close proximity to the cultivated sites were selected randomly for the study. Cultivated lands have been managed with organic fertilizers (n=4), synthetic fertilizers (n=3), or a combination of both types of fertilizers (n=3). Farmers used poultry manure, cattle manure, and compost as organic fertilizers. They applied fertilizer mixtures containing urea, triple superphosphate, and murate of potash as synthetic fertilizers. All farmers applied agrochemicals to control pests and diseases. In cultivated fields, crops such as leafy vegetables, capsicum, okra, long bean, bitter guard, snake guard, eggplant, and pumpkin had been cultivated

as mixed crops over the years at small scale (land extent <1/4 acre). Two locations were selected from each site for *in-situ* CDP estimation. Soil samples were collected from each sampling site at 0-15 cm depth in three replicates for soil analysis and laboratory incubation study (*ex-situ* CDP estimation). All the laboratory analyses were performed at the Department of Soil Science, University of Peradeniya, Sri Lanka.

Soil samples were air-dried and passed through a 2 mm sieve. The pH was measured in a 1:5 soil:water ratio. The organic C content was measured using Walkley and Black method (Nelson and Sommers, 1996) and the active-C content was measured according to the permanganate oxidizable carbon (POXC) method described by Weil *et al.* (2003)*.*

Soil incubation experiment

Air-dried and sieved soil samples were moistened with water to achieve a 25% (w/w) moisture level and incubated for three days without allowing it to dry. A quarter of cellulose filter paper (Whatmann® No.1) was placed in a sterilized petri-dish, and moist soil was packed to the petri dish and sealed well with parafilm. Three replicates were prepared for each soil sample, and the soil-filled Petri-dishes were kept under dark condition at room temperature. After two weeks, images of partially decomposed cellulose filter papers in Petri-dishes were captured using a camera. An image of a non-degraded filter paper was also captured to use as a control in image analysis. Image analysis was performed using Image-J® software. The percentage area of filter paper decomposed was estimated based on the size of the remaining piece and level of discoloration. The average decomposition percentage was calculated for filter papers incubated with uncultivated soil (Decomposition$_{uncultivated}$). Decomposition of filter paper incubated with cultivated soil (Decomposition$_{cultivated}$) in comparison to Decomposition$_{uncultivated}$ was calculated and used in statistical analysis.

In-situ test with litter bags

In-situ testing of CDP was determined employing the litter bag technique (Fekete *et al.*, 2008). Litter bags of 20 cm × 7 cm size were prepared using Nylon shade mesh (50% shade). Each bag was filled with 100 g of cotton wool (TAS MED® absorbent cotton wool for surgical purposes). The bags were buried at an angle at 10 – 15 cm depth to avoid excessive damping around litter bags. Litter bags were placed as two replicates per site.

The same procedure was repeated with placing cellulose filter papers (Whatmann® No.1) weighing 1.648 g per litter bag. Litter bags containing cellulose filter papers and cotton wool were recovered at 4 and 6 weeks after placement in soil, respectively. The material remaining in the litter bag was recovered and carefully washed to remove soil particles with density separation. The cleaned material was oven-dried at 60 °C until a constant weight was obtained (about 24 h).

Statistical analysis

Data were analyzed using the Analysis of Variance with General Linear Model (GLM) procedure to test the significance of land management using uncultivated, cultivated with organic fertilizers, cultivated with synthetic fertilizers, and cultivated with organic and synthetic fertilizers as the categories at *p*=0.05. When the effect of land management was significant (*p*<0.05), the mean comparison was performed using the least significant difference (LSD) method at P=0.05. Correlation between measured parameters was analyzed using Pearsons's correlation technique at *p*=0.05. Statistical analyses were performed using SAS® University edition software.

RESULTS AND DISCUSSION

Soils in the studied fields were slightly acidic and had relatively high organic C content (Table 1). According to the recently developed soil organic C map of Sri Lanka, the organic C levels in 0-30 cm depth range from 0.05 to 7.4% (Mishra & Mapa, 2019). The same study reported that the country average for soils cultivated with annual crops is 1.5% (Mishra & Mapa, 2019). The high organic C content support improvement of soil structure, reduction in bulk density, and increase in soil biological properties, leading to higher soil fertility (Lal, 2003). Soils under vegetable cultivation had relatively less organic C content compared to uncultivated soils, but the difference was significant only in soils managed with organic fertilizers alone. Fontaine *et al.*, (2004) observed that the addition of organic C accelerated the decomposition of SOM. They emphasized that competition among microorganisms for nutrient and energy sources plays an important role in organic C decomposition, and soil organic C losses increase when microbial communities are nutrient-limited. Hence, the addition of organic materials may not necessarily improve C storage (Fontaine *et al.*, 2004; Blagodatskaya *et al.*, 2014). The observed active-C contents in the present study are in the range generally reported for agricultural soils (Weil *et al.*, 2003).

Table 1. Characteristics of soils form uncultivated lands and vegetable cultivated lands managed with three different input use systems as organic fertilizers alone, synthetic fertilizers alone and a combination of organic and synthetic fertilizers

Land management category	pH	Organic C (%)	Active-C (mg/kg)
Uncultivated	4.56 ± 0.108 [a]	4.4 ± 0.11 [a]	315 ± 31.4 [a]
Cultivated			
Organic fertilizer	5.11 ± 0.359 [a]	3.5 ± 0.36 [b]	274 ± 35.9 [a]
Synthetic fertilizer	5.33 ± 0.547 [a]	3.6 ± 0.15 [ab]	293 ± 45.7 [a]
Organic+Synthetic fertilizers	5.33 ± 0.121 [a]	4.1 ± 0.10 [ab]	359 ± 50.5 [a]
p value[1]	NS	**	NS

[1] NS denotes means are not significantly different and * indicates means are significantly different at $p = 0.05$ for the measured property between different land management categories. Means are presented with ±standard error. Means followed by the same letter in a given column are not significantly different.

Ex-situ analysis of cellulose decomposition potential

In the soil incubation study, CDP was significantly affected ($P<0.0001$) by land management. The percentage area loss in filter papers due to the decomposition ranged from 3 to 99% by the end of two weeks, with the highest variability observed in soils from lands that received both organic and synthetic fertilizers (3 to 88%). The highest CDP was observed in lands cultivated with synthetic fertilizer inputs alone. It was nearly 9-folds higher than the decomposition in uncultivated soil (Figure 1). The cultivated soils managed with organic fertilizers alone had much similar CDP to natural vegetation. An increase in C mineralization with the increase in mineral nutrient availability has been reported previously (Strickland *et al.*, 2010). The filter paper assay was easy to perform and can be used to visualize data effectively (Figure 2).
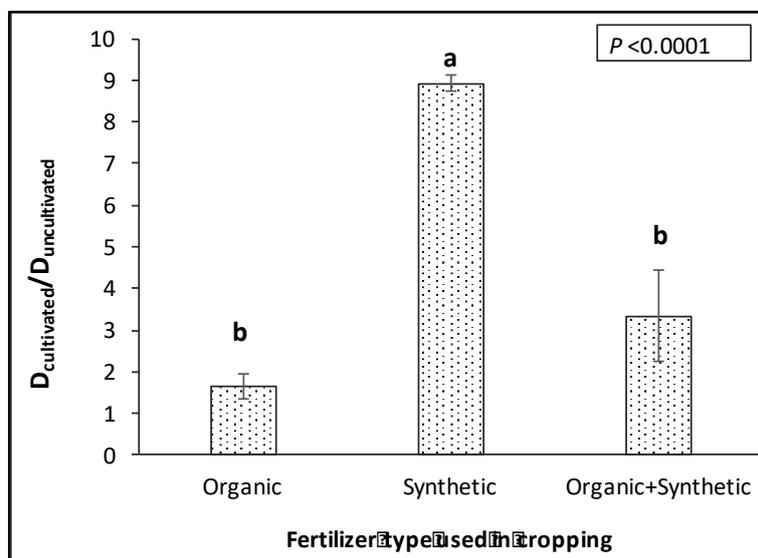

Figure 1. Decomposition status of cellulose filter papers incubated with soils collected from vegetable cultivated lands managed with organic and/or synthetic fertilizers in comparison to soil from uncultivated land (under natural vegetation) in the laboratory incubation experiment. Error bars indicate standard error. *p*<0.05 indicate that the effect of land management is significant. Means followed by the same letter are not significantly different at *p*=0.05.
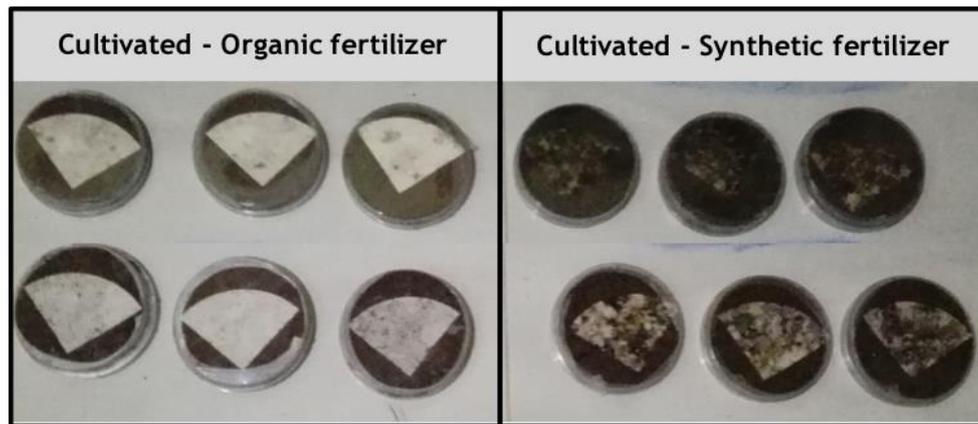
Figure 2. Status of filter paper degradation in two land management systems (cultivated with organic fertilizers alone and cultivated with synthetic fertilizers alone) at the end of two weeks of incubation.

In soil, there are r-strategist and K-strategist cellulose decomposers (Fontaine *et al.*, 2004; Blagodatskaya *et al.*, 2014). When a cellulose substrate is added to the soil, the fast-growing r-strategist cellulose decomposers would respond. They could express their full growth potential when mineral nutrients are not limiting (Fontaine *et al.*, 2004). On the other hand, K-strategist cellulose decomposers may dominate cellulolytic microbial communities in soils that receive high organic inputs with having low mineral nutrients (Fontaine *et al.*, 2004). Hence in soils managed with organic fertilizers alone, K-strategists get stimulated by cellulose inputs. On the other hand, r-strategist cellulose decomposers would respond to added cellulose in the soils applied with synthetic fertilizers alone. Therefore, the difference in CDP can be attributed to differences in the diversity of cellulolytic microbial communities in soil. The dominance of r-strategists and K-strategists in decomposers and functioning of microbial interactions affect soil organic-C content in the long run (Fontaine *et al.*, 2004; Blagodatskaya *et al.*, 2014). Stimulation of K-strategist cellulose decomposers increases the decomposition of soil organic C (Fontaine *et al.*, 2004). Therefore, soil exhibiting a high response of cellulolytic microorganisms (mainly r-strategists) to freshly added cellulose may still maintain high soil organic C content in the long run. Differences in CDP concerning land use/ management practices have been reported previously (Strickland *et al.*, 2010; Asigbaase *et al.*, 2020).

In-Situ analysis of cellulose decomposition potential

Interestingly, the effect of land management on CDP tested using cellulose filter papers and cotton wool as substrates were not significant ($p>0.05$) under field conditions. In general, the decomposition rate of cellulose filter paper was higher than that of cotton wool and ranged from 13 to 100% and 61 to 65% over one month, respectively (Figure 3). Of the total, 44% of samples exhibited over 80% decomposition of cellulose filter papers during one month period. From these samples, 75% were from cultivated fields. The results from *ex-situ* and *in-situ* analysis of CDP did not have a significant correlation ($P>0.05$). It may be partly because litter bags retrieved from soil much late to observe treatment effects. In the *ex-situ* analysis, the samples were observed two weeks after initiating incubation. Time allowed for decomposition is an important factor influencing the results of CMD studies (Fekete *et al.*, 2008; Dumitrache *et al.*, 2013). Kriauciuniene *et al.* (2012) studied litter decomposition from 2.5 to 26 months after placement in soil, whereas Fekete *et al.* (2008) studied the decomposition of litter and cotton wool over 3 to 12 months after placing in the soil. Fekete *et al.* (2008) observed that 59% of cotton wool was degraded in 3 months in the forested ecosystem in North-Eastern Hungary. In the present study, cotton-wool degradation one month after placement in soil ranged from 61-65%, indicating rapid decomposition in tropical climatic conditions. Therefore, we suggest periodical observations (e.g. weekly) within one month period on CDP when performing in-situ analysis in the future to determine the best observation window under humid tropical environments.

Mass balancing approach with litter bag studies is a comparatively easy approach to study CDP in soils though it has limitations (Boulton and Boon, 1991; van Gestel *et al.*, 2003; Fekete *et al.*, 2008;

Kriauciuniene *et al.*, 2012). One major drawback is the creation of artificial conditions such as excessive damping around the litter bag when placed in soil (Van Gestel *et al.*, 2003). In the present study, the litter bags were placed in the soil at an angle to minimize any damping effect.
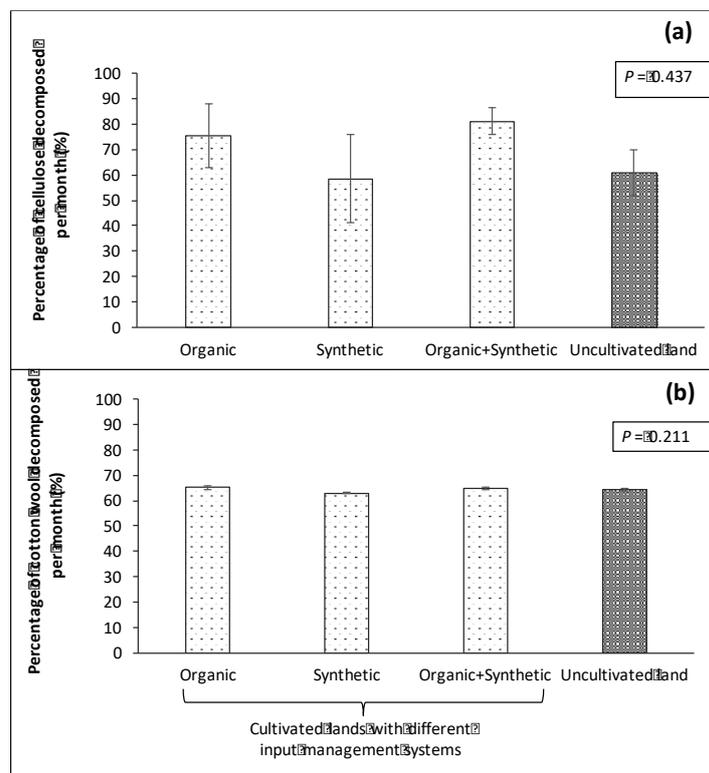

Figure 3. Decomposition rate (mass loss in grams per 100 g of material placed in soil for one month duration) of cellulose (a) and cotton-wool (b) in litterbags placed in vegetable cultivated and uncultivated lands at 10-15 cm soil depth. Error bars indicate standard error. *P* >0.05 indicate that treatment effect is not significant.

During the study period, there were frequent rainfall conditions, which is common in the Wet-zone of Sri Lanka (Punyawardena *et al.*, 2003). Soil moisture level and temperature regime are vital for cellulolytic microbial activity (Fekete *et al.*, 2008; Hossain *et al.*, 2017). Therefore, differences in soil moisture status and thermal conductance may also have contributed to the differences in CDP observed *in-situ vs. ex-situ*.

The composition of substrates affects their decomposition characteristics in soil (Fekete *et al.*, 2008; Kriauciuniene *et al.*, 2012). Cellulose cotton wool has been used previously in studying CMD and found to decompose faster than leaf litter owing to its homogenous structure and composition (Fekete *et al.*, 2008). In general, surgical cotton wool used in the present study is composed of 98% cellulose in about 60% crystalline structure and fringed fibril morphology (Gupta, 2013). Whatman® cellulose filter papers contain 98% cellulose though the fiber distribution, pores, and channels are highly heterogeneous (Dumitrache *et al.*, 2013; Costa *et al.*, 2014). Although both substrates used in the present study have cellulose as the main constituent, cotton cellulose differs from cellulose present in the filter paper primarily by having a higher degree of polymerization and crystallinity. The physical structure of the materials affects the accessibility for microbial penetration and subsequent colonization. The accessible surface area of cellulolytic material determines its decomposition rate (Dumitrache *et al.*, 2013). The structural differences in cotton wool and filter paper may have contributed to observed differences in CDP. Further, cotton wool used for medical purposes has high hydrophilic characteristics with the ability to imbibe up to 30% moisture by mass (Gupta, 2013). Therefore, the microclimate in the litter bag containing cotton wool would be different from that containing cellulose filter papers.

A significant correlation between litter decomposition and soil characters like pH and moisture content has been reported previously (Asigbaase *et al.*, 2020). However, in the present

study, CDP had no significant correlations ($p>0.05$) with the measured soil parameters (pH, organic C, and active-C). It could be because the soils were generally high in organic C contents and had less variability of pH and active-C contents (Table 1). While soil organic C in the studied soils ranged from 2.3 to 4.6% the CDP assessed based on the decomposition of cellulose filter papers ranged from 3 to 98% during the two-week period in *ex-situ* conditions and 14 to 100% during the four-week period in *in-situ*. Although the pH and organic C varied in narrow ranges, it is interesting to note that CDP showed very high variability. It should be due to the difference in the diversity of microbial communities in these soils.

CONCLUSIONS

In the studied region, vegetable cultivation caused a decline in soil organic C content to variable degrees depending on nutrient inputs used. Vegetable cultivation affected CDP differently depending on the nature of cellulose input and the history of fertilizer management. Carbon mineralization and decomposition efficiency, reflected by CDP were highest in vegetable cultivated soils managed with synthetic fertilizer inputs alone. *Ex-situ* analysis of CDP using cellulose filter papers could effectively be used to demonstrate the differences in microbial activity in soils. From the results, it is inferred that the nutrient management practices changed the diversity of the heterotrophic microbial community. It would be interesting to further investigate whether organic fertilizer usage leads to the dominance of K-strategist decomposers compared to other fertilizer management regimes under vegetable cultivation. It is suggested to periodically analyze the CDP over one month to identify the best observation point for in-situ determination of CDP in a humid tropical climate as in Kegalle, Sri Lanka.

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