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Full Length Research Paper

Responses of soil microbial biomass carbon to tillage and fertilizer types in maize cultivation in Buea, Cameroon

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Although soil microbial biomass (MBC) comprises less than 5% of soil organic matter, it responds rapidly to changes in soil management practices and, therefore, is generally used as an early indicators of changes in soil carbon. The objective of this study was to evaluate the effects of tillage practices (conventional tillage and no-tillage) and fertilizer types (synthetic, organic, and no fertilizer) on soil MBC. The field experiment, located in Buea, was arranged in a split-plot design with three replications and had tillage systems as main plots and fertilizer types as sub-plots. Soil samples were collected at 0–15 cm depth at an interval of 4 (early season), 8 (mid-season) and 12 (late season) weeks during the 2020 and 2021 minor and major growing seasons respectively, for the determination of soil MBC by the chloroform fumigation and extraction method. The findings of the study showed that the main effect of tillage practice and fertilizer types was nonsignificant (p>0.05) in the 2020 and 2021 study season throughout the sampling period. Plots under zero tillage with control experiments (No.Till:CON) recorded the highest soil MBC in the 2020 season (201 mg/kg) while in the 2021 season, plots under zero tillage with organic fertilization (No.Till:ORG) recorded the highest (400.4 mg/kg) soil MBC. Soil MBC was higher in the 2021 season than in the 2020 season. These findings suggest that the use of compost in combination with either conventional tillage or no-tillage in farms in the study area could potentially enhance soil MBC.

Key words: Tillage, fertilizer type, microbial biomass carbon, carbon sequestration.

INTRODUCTION

Soil organic carbon (SOC) in the top 100-cm soil layer holds about two times as much carbon (C) than is in the atmospheric pool, making the soil the largest C pool in the terrestrial biosphere (Chen et al., 2015). According to

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Jagadamma and Lal (2018), the C sink capacity of the earth's soil is about 1 Pg C year^{-1}. This means that relatively small change in SOC can have a significant impact on atmospheric CO_2 level (Lal et al., 2007). Currently, there is a strong interest in sequestrating C in soils to help decrease atmospheric CO_2 level (Liang et al., 2021). Agroecosystems, which represent large portions of terrestrial ecosystems, if well managed, can provide an opportunity to increase soil C pools and reduce atmospheric CO_2. In agroecosystems, enhanced C sequestration in agricultural soils does not only have the potential to help reduce atmospheric CO_2 concentrations (Sperow et al., 2003), but also promotes the productivity and sustainability of agricultural systems since increased soil C sequestration in agricultural soils improves soil quality, increases soil productivity, and reduces risk of soil erosion and sedimentation (Lal et al., 2007). In Africa, crop productivity is most affected by the adverse impacts of climate change. Therefore, more studies are needed that address how to promote enhanced C sequestration in cropland ecosystems.

Although soil microbial biomass comprises less than 5% of organic matter, it responds rapidly to changes in soil management and can be used as early indicators of changes in soil C and C sequestration (Kallenbach and Grandy, 2011). In agroecosystems, soil management practices such as tillage systems and fertilizer types affect soil microbial biomass. Tillage operations, which are the ploughing of the soil to prepare it for sowing, can decrease soil microbial activity and organic matter (Mohammadi et al., 2012). Continuous use of conventional tillage (CT) system influences the physical and chemical properties of soils which in turn directly affect the biological activities of the soil (Lupwayi et al., 2012). Tillage mechanically disturbs soil aggregates; increases soil aeration, and accelerate soil organic matter decomposition by soil micro-organisms. On the other hand, minimum and no-tillage can improve soil physical properties as macro-pore structure, aggregate stability, nutrients availability, and enhance the diversity and activity of microbial populations. In a four-year study conducted by Lupwayi et al. (2012) in Saskatchewan, Canada, authors noted that zero tillage increased soil microbial biomass (MBC) by 30 to 102% and tended to increase bacterial functional diversity under corn cultivation. Similarly, Wright et al. (2015) also noted that soil MBC and were often highest under zero tillage and minimum tillage in surface soils in tropical soils under corn. According, Wright et al. (2015), conventional tillage recorded the lowest soil MBC during the period of the study.

The application of fertilizer to provide nutrients for crops can influence soil chemical properties, and microbial biomass and activity. For example, the application of organic fertilizer enhances soil microbial activity, through improving activity of soil enzymes and increasing soil microbial biomass (Nair and Ngouajio, 2012). Chu et al. (2007) in a study conducted to investigate soil MBC response to fertilization application types noted that organic fertilization had a significantly greater impact on the soil MBC and the activity of soil microbes compared with mineral fertilizers. In a recent study conducted in the Liaoning Province of China, Luo et al. (2015) shared similar results as their findings revealed that long-term organic fertilization greatly increased soil MBC, while synthetic fertilization reduced soil MBC. The authors concluded that organic fertilizer had a significantly greater impact on soil MBC under corn cultivation. Aside of its carbon sequestration benefits in the soil, soil microbial biomass (SMB) is an immediate sink of N, P and S (Dick, 1992); and it is an agent of nutrient transformation and pesticide degradation. Soil microbial biomass is, therefore, a fundamental component of nutrient cycling in agroecosystems.

Despite the multiple benefits of sequestering C in agricultural soils, the impacts of key soil management practices such as tillage and fertilization types on SMB is still under reported in many agro-ecological areas across Africa. Also, in most parts of Africa including Cameroon, farmers apply both inorganic and organic fertilizers without taking into consideration their effects on SMB. This, sometimes, leads to poor planning and management of soil amendments, which in turn results in the reduction of farm productivity since SMB plays an important role in soil organic matter decomposition and nutrient cycling (Logah et al., 2010). One of the biggest challenges of agriculture in many parts of Africa is to find best soil management practices that guarantees food production and environmental sustainability, while minimizing the vulnerability of the farming system to the impacts of climate change (Jouzi et al., 2019). Therefore, localized studies on the role of soil management practices on SMB, which can be used as an indicator of C sequestration and nutrient cycling in agroecosystems, are more than needed. There is need to document the impacts of soil management practices on microbial biomass carbon in Buea, Cameroon. This study was designed to bridge this knowledge gap. We hypothesized that tillage and fertilizer types have a significant effect on microbial biomass carbon. To test this hypothesis, we investigated the response of soil microbial biomass carbon (MBC) to tillage regime (till vs no-till) and soil amendment types (that is, synthetic fertilizer, organic fertilizer, and unfertilized control) under maize cultivation in the 2020 and 2021 growing seasons in the Buea Municipality, Southwest Region of Cameroon.

MATERIALS AND METHODS

Description of study area

The field experiment was conducted at the research farm of the Department of Environmental Science, University of Buea. The University of Buea is located between latitudes 4º3'N and 4°12'N and longitude 9°12'E and 9°20'E (Ngosong et al., 2019). Buea, which is the capital of the southwest region of Cameroon, lies along the eastern slopes of Mount Cameroon, bounded to the north by a

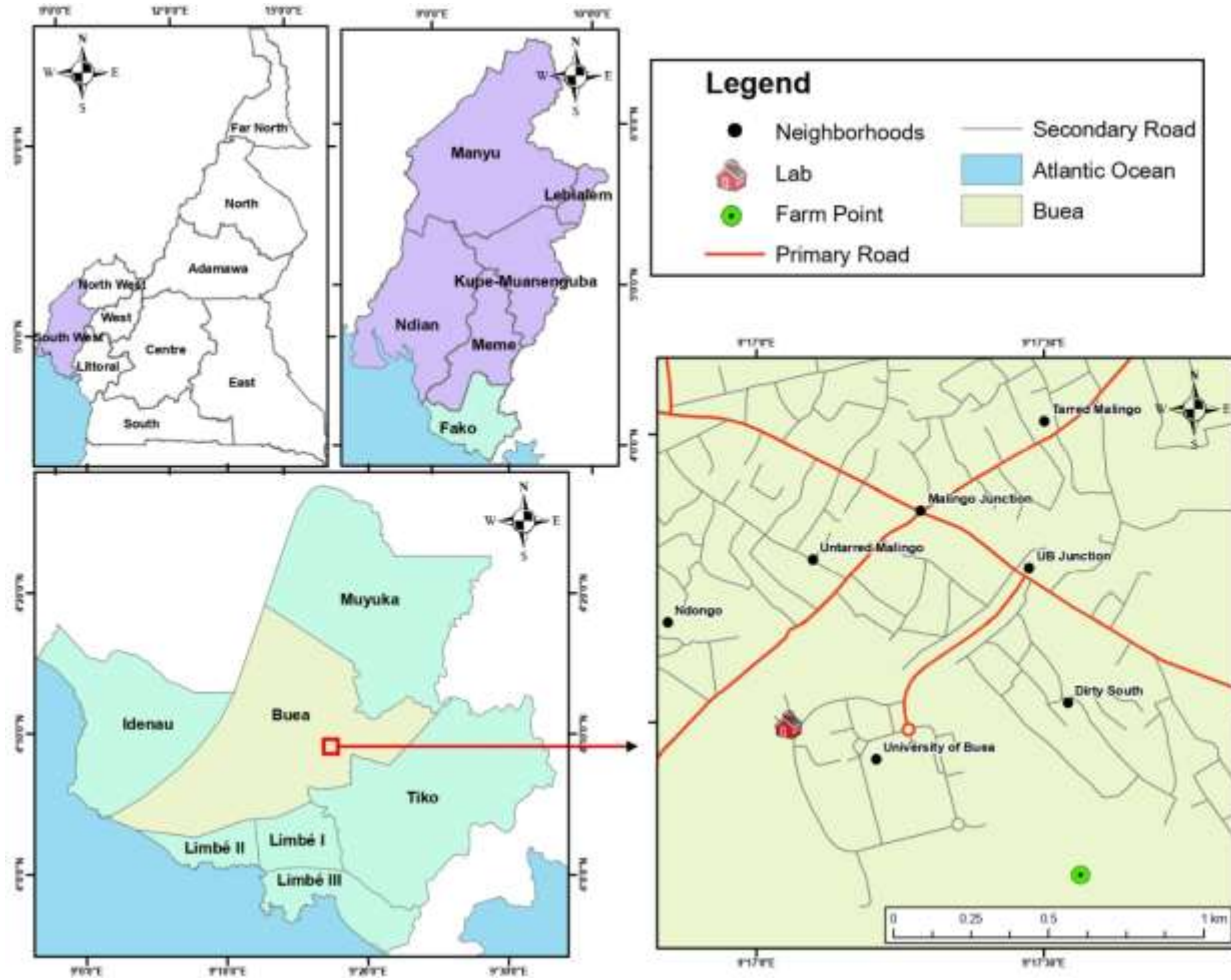

Figure 1. Map of the study area.
Source: Authors

tropical forest on the slope of mount Cameroon (4,100 m a.s.l.). The mountain range extends to the beautiful sandy beaches of the Atlantic Ocean. The town also shares boundaries with other major towns like the city of Limbe to the south-west, Tiko municipality to the southeast, Muyuka municipality to the east, and Idenau district to the west (Figure 1).

Buea has an equatorial climate with two major seasons; a rainy season, which runs from March to October; and a dry season, from November to February. Temperature ranges between 20 °C to 28 °C, while annual rainfall ranges between 3000 mm and 5000 mm. The equatorial climate of the city makes it possible to have two maize growing seasons in Buea; the major growing season from March to July and the minor growing season from September to November (Ako, 2011).

The soils of this region are developed from the weathering of a basaltic parent rock. These soils have been intensely weathered in some areas to produce well drained to clayey reddish brown and yellowish soils, which are over 10 m thick. Yet in other areas, the soils are well drained, relatively young black soils developed from protracted weathering of basaltic rock and pahoehoe lava flows (Ako, 2011). Buea soils are very rich in nutrients and support the cultivation of various crops such as maize, tomatoes, cabbage, okra, pepper, corn, cocoyam, yams, cassava, plantains, beans, vegetables and even some cash crops such as palm trees, cocoa, and bananas (Ngosong et al., 2019).

Experimental design and treatments

The field experiment was conducted during the 2020 minor growing season (Late September to Late December 2020) and 2021 major growing seasons (Late March to early July 2021). The field experiment was a split-plot design with three replications (Figure 2). The main plot factors were tillage practices (that is, conventional tillage and no-till) and the sub-plots were fertilizer types (that is, organic, inorganic, and no amendment used as control). Within each replicate, a 2-m buffer was kept between the main plots and the sub-plots and a 5-m buffer to separate the blocks or repetitions.

The tilling systems evaluated was no till and conventional till. Two fertilizer types (composted municipal solid waste and Urea) and a control (no amendment) were adopted. A nitrogen fertilizer application rate of 100 kg/ha was adopted based on the recommendations of Ngosong et al. (2019) on best N application rate in volcanic soils along the slopes of Mount Cameroon. Prior to applying the compost, samples were taken for analysis for the determination of N, P and K concentration in the compost manure.

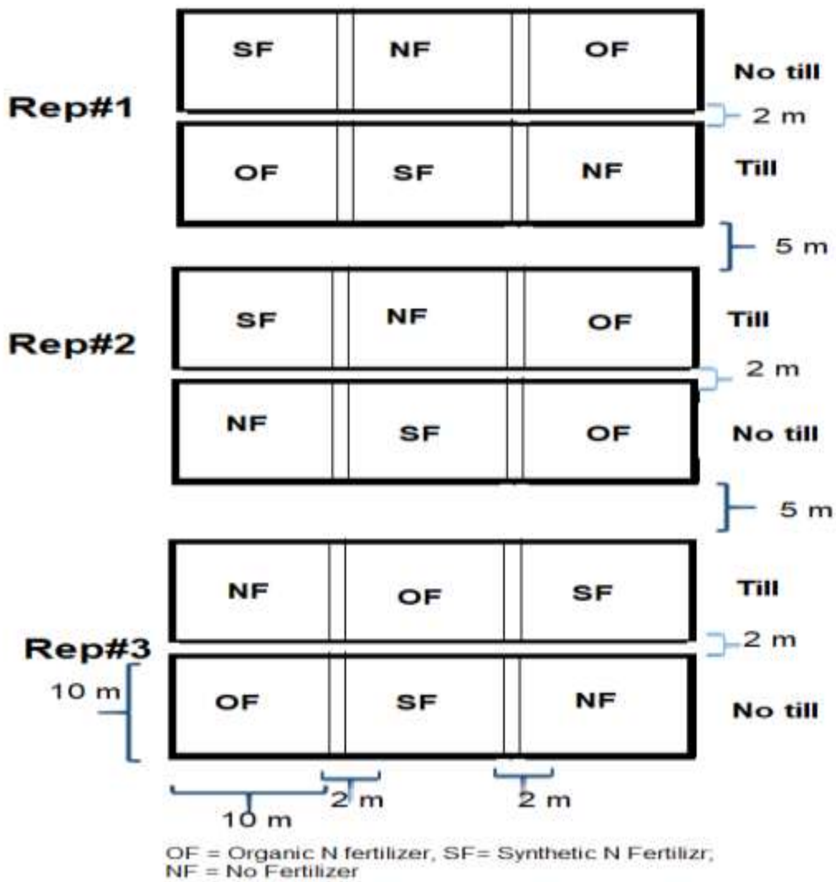

Figure 2. Experimental layout in a split plot design.
Source: Authors

Based on the N content (11%) of the compost samples analyzed, we applied compost at the rate 2.275 kg per plot of 25 m^2 to provide 100 kg/ha equivalence application of N as recommended by Ngosong et al. (2019). For Urea, with a known concentration of N (46%), we applied it at the rate of 0.55 kg per plot to provide the equivalence of 100 kg/ha. Both fertilizers were applied on the same day, one month after planting in both seasons.

The cultivar of the test crop was hybrid maize CMS 8704 cultivar obtained from the Regional Delegation of Agriculture in the Southwest Region of Cameroon. A seeding rate of 45.55 kg/ha was adopted. Based on this seeding rate, 114 g of maize seeds were planted within each sub plot of 25 m^2. Each maize stand had three seeds and a spacing distance of 80 cm was allowed between each maize stand and the next as recommended by FAO. Each sub plot had 36 maize stands in total. In situations where the maize did not germinate well within one week, seeds were replanted. On farm activities such as weeding was applied for all the plots throughout the growing season according.

Plot preparation

The study site was cleared on 2nd September 2020 for planting in the 2020 minor growing season and on 10th March 2021 in the 2021 major growing season. After clearing the field, all plant residues were removed from the plots the same way it is practiced by small holder farmers in the study area. A measuring tape was used to split the study site into 18 sub-plots of 25 m^2 (5 m x 5 m), each. A sawn timber of 1.5 m was used to demarcate the plot boundaries within the study site. Properly labelled plywood measuring 10 cm by 15 cm was placed at the center of the sub-plots to show the locations of the main plots and sub-plots. Conventional tillage was applied on the tilled plots using a hoe during all study seasons.

Initial soil sampling and analysis

Initial soil samples were randomly collected for the study on 14th of September 2020 to determine the physico-chemical parameters of the soil of the study site. A soil auger was used to collect 36 core samples at a depth of 0-15 cm from the study plot. Samples were air dried at the Department of Environmental Science Laboratory for 14 days, after which they were bulked to form one composite sample for analysis for soil physico-chemical parameters, such as soil texture, bulk density, pH, electrical conductivity, soil Organic C, total nitrogen, calcium, magnesium, potassium, sodium, available phosphorus and cation exchange capacity. Soil sample analysis was conducted at the Laboratory of Faculty of Agriculture, University of Dschang in Cameroon.

Measurement of soil Microbial C

Ten plants were selected at random from the middle rows of each plot. Soil samples were taken from the base of each plant at a depth of 0–15 cm (McClaran et al., 2008) using a hand auger. The

10 auger soil samples were then composited together (bulked) to form a representative sample for each plot in both growing seasons. Three samplings were made during each season at intervals of 4, 8 and 12 weeks during each growing season. Soil samples were kept in an ice cooler to halt any microbial activity and transported from the field to the Laboratory prior to analysis. The analysis of the soil MBC was determined at the laboratory of Faculty of Agriculture at the University of Dschang.

Soil MBC in the samples was determined using the chloroform fumigation and extraction method (FE) as described by Ladd and Amato (1989). Following this method, ten grams of field moist soil sample, after passing through a 4-mm mesh, were put in a crucible and placed in a desiccator. A shallow dish containing 30 ml of alcohol-free chloroform was placed by it. A crucible containing a control sample (10 g) was placed in a separate desiccator without chloroform. The desiccators were covered and allowed to stand at room temperature for 5 days (Ladd and Amato, 1989).

Immediately after fumigation, 50 ml of 0.5 MK_2SO_4 solutions was added to the soil samples to extract MBC from the lysed microorganisms. The amount of MBC in the extract was determined using the colorimetric method. An aliquot (5 mL) of the extract was pipetted into a 250-mL Erlenmeyer flask. To this, 5ml of 1.0 N (0.1667 M) potassium dichromate and 10 mL of concentrated sulphuric acid was added. The resulting solution was allowed to cool for 30 min after which 10 mL of distilled water was added. A standard series was developed concurrently with C concentrations ranging from 0, 2.5, 5.0, 7.5, 10.0-mg C mL^{-1} C. These concentrations were obtained when volumes of 0, 5, 10, 15 and 20 ml of a 50 mg C mL^{-1} stock was pipetted into labelled 100-mL volumetric flasks and made up to the mark with distilled water. The absorbance of the standard and sample solutions was read on a Spectronic 21D spectrophotometer at a wavelength of 600 nm.

A standard curve was obtained by plotting absorbance values of the standard solutions against their corresponding concentrations. Extracted C concentration of the samples was determined from the standard curve. For biomass C calculations, k -factors of 0.35 (Sparling et al., 1990) was used. The following equations (Sparling and West, 1988) were used to estimate the microbial C from the extracted C (Equation 1).

$$\text{Microbial C (mg)} = Ec/k \quad (1)$$

Where Ec = the extracted carbon produced following fumigation; k = the fraction of the killed biomass extracted as carbon or nitrogen under standardized conditions.

Statistical data analysis

After obtaining the data of soil MBC for all plots, R package Agricola was used to analyze the data for differences in treatments. The UNIVARIATE procedure was used to test the data and residuals for the assumption of normality to carry out a descriptive statistic and to draw graphs illustrating the effects of tillage, treatment and sampling period on soil MBC. An ANOVA test on R studio was conducted to test the effects of tillage and treatment on soil MBC. Soil MBC data was analyzed as a randomized complete block design (RCBD) using two-way ANOVA. Separation of means was done using the Tukey-Kramer adjustment least significant difference (LSD) method at alpha level of significance of 0.05 (Logah et al., 2010).

RESULTS

Physico-Chemical properties of the study site and compost analysis

The results of the physico-chemical properties of the study site and nutrient content of the compost are shown in Tables 1 and 2, respectively.

Impacts of tillage and fertilizer types on soil Microbial Biomass Carbon (MBC)

In the early growing season of 2020, tilled plots under control experiment (Till:CON) recorded the highest soil MBC (200.5 mg/kg), while the lowest (116.1 mg/kg) was recorded in not tilled plots under organic fertilization (No.Till:ORG).

In mid-growing season, the highest soil MBC (257.6 mg/kg) was recorded in plots under zero tillage with control experiment (No.Till:CON) and the lowest (182.mg/kg) was recorded in tilled plots under organic fertilization (Till:ORG).

During the late season sampling, the highest (261.6 mg/kg) soil MBC was recorded in No.Till:ORG, while the least (161.9 mg/kg) was recorded in plots under zero tillage with synthetic fertilization (No.Till:SYN). Overall seasonal analysis in the 2020 study season showed that No.Till.CON and No.Till:ORG recorded the highest MBC (201mg/kg and 200mg/kg respectively) while Till:ORG recorded the lowest (168 mg/kg) (Figure 3). Detailed data are in Appendix 1.

Results of this study also reveal that tillage and fertilizer types had no significant effect (P>0.05) effects on soil MBC in the early, mid and late season sampling in 2020 (Table 3). The means of soil MBC were statistically the same in both tillage and fertilizer application systems in these sampling periods (Figure 3). The overall growing season results for the three-sampling period showed that tillage and fertilizer types had no significant effect (P>0.05) on soil MBC. The interaction level means were also the same in both tillage practices and fertilizer application types (Figure 3).

During the 2021 study seasons, early season samples showed that, Till:ORG recorded the highest soil MBC (357.2 mg/kg) while the lowest (221.6 mg/kg) was generated in Till:CON. In the mid growing season, the highest soil MBC (385.5 mg/kg) was recorded in No.Till:ORG and the lowest (245.8 mg/kg) was recorded in Till:SYN. During the late season sampling, the highest (486.6 mg/kg) soil MBC was recorded in No.Till:ORG while the least (199.5 mg/kg) was recorded in Till:SYN. The overall seasonal results showed that the highest mean soil MBC occurred in No.Till.ORG (400.4 mg/kg), while the least occurred in Till.SYN (230.3 mg/kg) (Figure 4). Detailed results are shown in Appendix 2.

In the 2021 study season, the findings of this study revealed that tillage and fertilizer types had no significant effect (P>0.05) on soil MBC in the early, mid and late season sampling (Table 4). The means of soil MBC during the first sampling period were statistically the same in different tillage and fertilizer application systems (Figure 4). However, the means of soil MBC during the mid and late sampling period were statistically different in

Table 1: Physicochemical properties of the soil from the study site

Parameter	Unit of Measurement	Value
Sand	%	18
Silt	%	33
Clay	%	49
Electrical conductivity	ms/cm	0.04
Bulk density	g/cm^3	1.15
pH-H_2O (1:2.5)		5.8
pH-KCl (1:2.5)		4.7
Soil organic carbon	(%)	3
Total nitrogen	(%)	0.10
C/N		30
Calcium	(cmol/kg)	4.88
Magnesium	(cmol/kg)	3.44
Potassium	(cmol/kg)	4.50
Sodium	(cmol/kg)	0.01
Cation exchange capacity	(cmol/kg)	8.48
Available phosphorus	(mg/kg)	4.10

Source: Authors

Table 2. Results of NPK content of compost.

Parameter	% Content
Total Nitrogen	11
Total Phosphorus	0.24
Total Potassium	1.54

Source: Authors

plots under different tillage practices and fertilizer application systems as revealed by the LSD test. Overall growing season results for the three-sampling period showed that tillage and fertilizer types had no significant effect ($P>0.05$) on soil MBC (Table 4). However, the means of soil MBC in the different tillage practices and fertilizer types were not the same (Figure 3).

Findings of this study also revealed that there was a significant difference ($p<0.05$) in soil MBC in the first and second growing seasons of the study. Here, we noted that values of soil MBC were higher in the second growing season compared the first growing season.

DISCUSSION

Although there was no significant effects of tillage practice and fertilizer application types on soil MBC in both seasons, the study noted that No.Till:ORG recorded in the highest mean soil MBC in the first and second study season. Zero tillage leads to accumulation of higher concentration of organic C and microbial biomass C (Yeboah et al., 2016; Wright et al., 2015). The application of organic fertilizer in these plots under zero tillage also helped in the addition of C-rich organic compounds to the microbial communities (Knapp et al., 2010; Luo et al., 2014). Thus, this could be the reason for increased soil MBC in No.Till: ORG in this study. In a similar study in Iran conducted by Mohammadi et al. (2012), the authors also reported that the addition of organic manure increased soil MBC relative to synthetic fertilizer in plots under zero tillage. Especially in tropical climates, soil MBC is highest in the top 0–2.5 or 0–5 cm depths of undisturbed soil (Rai et al., 2018); therefore, limiting tillage can be a means to increase soil MBC in cropland ecosystems. Also, with the increasing cost of imported synthetic fertilization especially for small scale farmers in the tropics, using compost recycled from organic waste can reduce farmers cost in agriculture as well increase soil MBC in their farms, which helps in the long run sustainability of the farming systems. However, other trade-offs associated to the use of organic fertilizers (such as bulk and slow rate of reaction compared to synthetic fertilizers) needs to also be considered. These trade-offs can limit the application of organic fertilizer, especially in situations where farmer have long distance farms.

The authors also noted that soil MBC was significantly different ($P<0.05$) in the 2020 and 2021 study seasons. The means of soil MBC were higher in the 2021 study

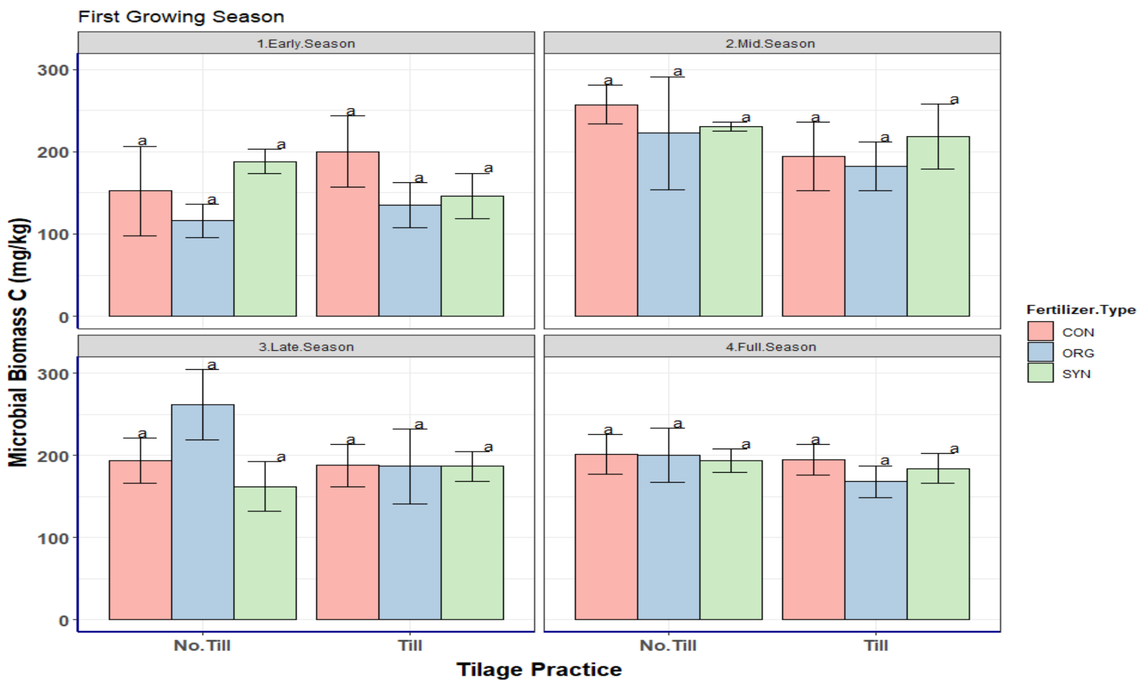

Figure 3. Mean Soil MBC in plots under different tillage and fertilizer types across sampling date in the first growing Season.
CON = Control, SYN=Synthetic Fertilizer, ORG=Organic Fertilizer, No Till=No Tillage Applied, Till: Conventional Tillage Applied.
Source: Authors

Table 3. ANOVA results on the effects of tillage and fertilizer type on soil MBC in the 2020 study season.

Variable	Df	Sum sq	Mean sq	F value	Pr(>F)
Early season					
Fertilizer type	2	8793	4396.6	1.2571	0.3194
Tillage practice	1	316	315.8	0.0903	0.7689
Fertilizer type: Tillage practice	2	6384	3192.1	0.9127	0.4276
Residuals	12	41968	3497.3		
Mid-season					
Fertilizer type	2	2097	1048.6	0.2214	0.8046
Tillage practice	1	6625	6624.8	1.3985	0.2599
Fertilizer type: Tillage practice	2	2004	1001.8	0.2115	0.8123
Residuals	12	56844	4737		
Late season					
Fertilizer type	2	2097	1048.6	0.2214	0.8046
Tillage practice	1	6625	6624.8	1.3985	0.2599
Fertilizer type: Tillage practice	2	2004	1001.8	0.2115	0.8123
Residuals	12	56844	4737		
Full season					
Fertilizer type	2	1724	861.9	0.1978	0.8212
Tillage practice	1	3551	3551.2	0.8148	0.3712
Fertilizer type: Tillage practice	2	1723	861.7	0.1977	0.8213
Residuals	48	209197	4358.3		

Source: Authors

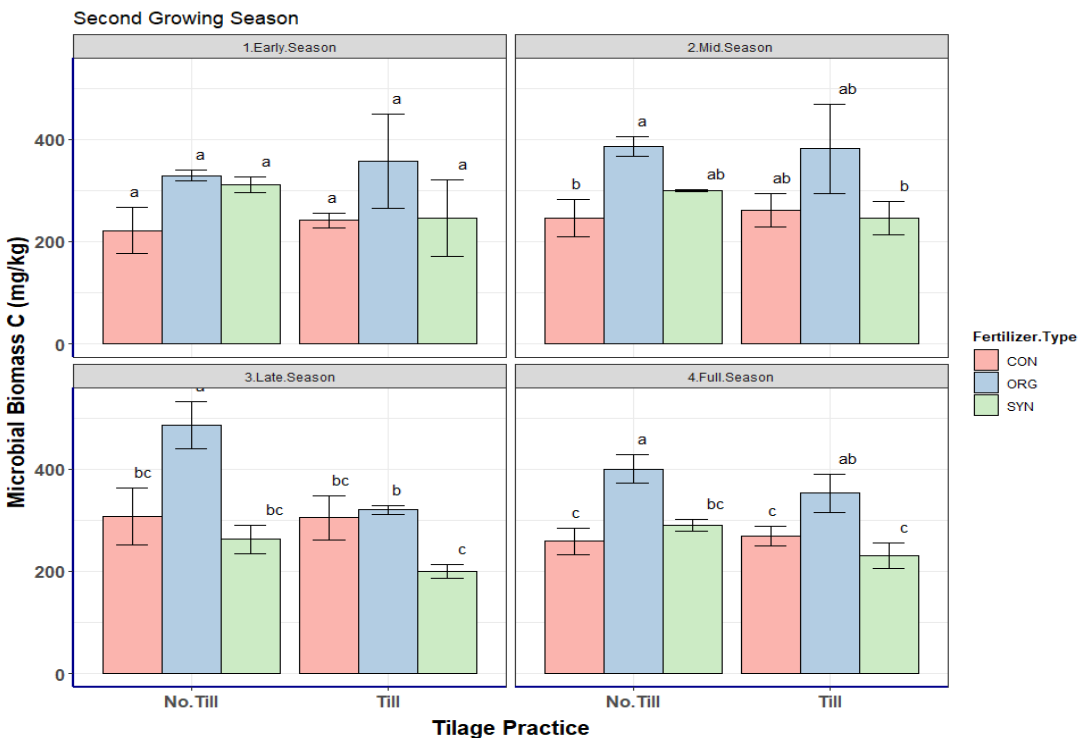

Figure 4. Mean Soil MBC in plots under different tillage and fertilizer types across sampling dates in the second growing Season.
Source: Authors

Table 4. ANOVA results on the effects of tillage and fertilizer type on soil MBC in the 2021 study seasons.

Variable	Df	Sum sq	Mean Sq	F value	Pr(>F)	
Early season						
Fertilizer type	2	37835	18917.3	2.2758	0.1452	
Tillage practice	1	156	155.6	0.0187	0.8934	
Fertilizer type :Tillage practice	2	7996	3997.8	0.4809	0.6296	
Residuals	12	99750	8312.5			
Mid-season						
Fertilizer type	2	59001	29500.4	5.0501	0.02563	*
Tillage practice	1	902	901.9	0.1544	0.70127	
Fertilizer type :Tillage practice	2	3679	1839.3	0.3149	0.73574	
Residuals	12	70099	5841.5			
Late season						
Fertilizer type	2	89420	44710	11.079	0.00188	**
Tillage practice	1	27085	27085	6.7112	0.02363	*
Fertilizer type : Tillage practice	2	20569	10284	2.5483	0.11957	
Residuals	12	48429	4036			
Full season						
Fertilizer type	2	157334	78667	13.097	2.89E-05	***
Tillage practice	1	14294	14294	2.3798	0.1295	
Fertilizer type: Tillage practice	2	12880	6440	1.0721	0.3503	
Residuals	48	288312	6007			

Source: Authors

season compared to the 2020 season. These differences may have occurred due to the differences in environmental conditions of rainfall, soil moisture and soil temperature across the two seasons. During the 2020 season, soil samples for this analysis were collected between late September and late December, a period characterized by a lower rainfall and higher atmospheric and soil temperatures. In the 2021 study season on the other hand, samples were collected between late March and early July, which corresponded to a typical rainy season period. Besides tillage practices and fertilizer application types, temperature and moisture predominantly determine the amount of microbial biomass in a soil (Wardle and Parkinson, 1990).

According to Kopittke et al. (2017), microbial biomass increases with increasing mean annual precipitation; however, it decreases with mean annual temperature increase above 20^{o}C in a semi-arid subtropical environment. Furthermore, seasonal fluctuations in soil microbial biomass occur due to changes in the number of substrates, temperature, and moisture. For example, Lynch and Panting (1982) found that the amount of microbial biomass reached a maximum around the time of maximum root biomass and thereafter declined.

Conclusion

This study has effectively documented main effects of tillage practices and fertilizer types on soil MBC under maize cultivation. The results show that the main effect of tillage practice and fertilizer types was insignificant (p>0.05) in the 2020 and 2021 study season. However, the mean values of soil MBC in different tillage and fertilizer application types were statistically the same in the 2020 season; while in the 2021 study season, the means were statistically different.

No.Till:CON and No.Till:ORG recorded the highest soil MBC in the 2020 season (201 and 200 mg/kg respectively) while in the 2021 season, No.Till:ORG recorded the highest (400.4 mg/kg) soil MBC. Soil MBC was higher in the 2021 season than in the 2020 season. Based on these findings, we recommend the use of minimum tillage and organic fertilizer application in farms around the study area to guarantee the maximum benefits of carbon sequestration like improved soil quality, increased soil productivity and reduced risk of soil erosion and sedimentation in farmlands around the study area.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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Appendix 1. Data summary (Mean, std, min, max) of soil MBC for the 2020 growing season.

Sampling period	Fertilizer type	Tillage practice	Mean (mg/kg)	Sd (mg/kg)	Min (mg/kg)	Max (mg/kg)
Early season	CON	Till	*200.5*	74.4	120.3	267.3
Early season	SYN	No till	188.4	25.4	162.7	213.5
Early season	CON	No till	152.3	95.0	49.5	236.9
Early season	SYN	Till	146.2	48.0	115.7	201.5
Early season	ORG	Till	135.1	47.5	80.5	166.1
Early season	ORG	No till	*116.1*	34.9	94.1	156.3
Mid-season	CON	No till	*257.6*	40.7	210.6	281.3
Mid-season	SYN	No till	230.3	9.5	223.0	241.1
Mid-season	ORG	No ill	222.6	119.4	104.5	343.3
Mid-season	SYN	Till	218.7	68.2	148.7	284.9
Mid-season	CON	Till	194.5	71.4	136.0	274.1
Mid-season	ORG	Till	*182.3*	51.6	150.1	241.8
Late season	ORG	No till	*261.6*	74.7	203.4	345.8
Late season	CON	No till	193.2	47.2	141.8	234.5
Late season	CON	Till	187.8	44.4	147.8	235.6
Late season	ORG	Till	186.6	78.5	133.1	276.7
Late season	SYN	Till	186.3	30.7	157.6	218.7
Late season	SYN	No till	*161.9*	52.3	129.8	222.2
Full season	CON	No till	*201.0*	73.1	49.5	281.3
Full season	ORG	No till	*200.1*	97.6	94.1	345.8
Full season	CON	Till	194.3	56.4	120.3	274.1
Full season	SYN	No till	193.5	42.0	129.8	241.1
Full season	SYN	Till	183.8	54.5	115.7	284.9
Full season	ORG	Till	*168.0*	58.2	80.5	276.7

CON = Control, SYN=Synthetic Fertilizer, ORG=Organic Fertilizer, No Till=No Tillage Applied, Till: Conventional Tillage Applied.

Appendix 2. Data summary (Mean, std, min, max) of soil MBC for the 2021 growing season.

Sampling period	Fertilizer type	Tillage practice	Mean (mg/kg)	Sd (mg/kg)	Min (mg/kg)	Max (mg/kg)
Early season	ORG	Till	*357.2*	158.8	215.7	529.0
Early season	ORG	No till	329.3	19.5	312.1	350.5
Early season	SYN	No till	310.7	25.8	282.5	333.0
Early season	SYN	Till	245.4	129.8	101.9	354.4
Early season	CON	Till	241.3	25.0	215.0	264.8
Early season	CON	No till	*221.6*	78.3	167.0	311.4
Mid-season	ORG	No till	*385.5*	33.0	355.0	420.4
Mid-season	ORG	Till	381.1	152.8	252.3	550.0
Mid-season	SYN	No till	298.9	3.3	296.0	302.4
Mid-season	CON	Till	261.0	57.6	212.7	324.8
Mid-season	CON	No till	246.1	62.8	200.1	317.6
Mid-season	SYN	Till	*245.8*	57.8	205.5	312.0
Late season	ORG	No till	*486.6*	79.5	398.1	552.1
Late season	ORG	Till	319.9	14.2	304.9	333.1
Late season	CON	No till	307.8	96.4	224.1	413.2
Late season	CON	Till	304.7	74.7	220.4	362.6
Late season	SYN	No till	262.5	48.2	218.4	313.9
Late season	SYN	Till	*199.5*	22.4	174.2	216.6
Full season	ORG	No till	*400.4*	81.9	312.1	552.1
Full season	ORG	Till	352.7	113.6	215.7	550.0
Full season	SYN	No till	290.7	35.0	218.4	333.0
Full season	CON	Till	269.0	56.3	212.7	362.6
Full season	CON	No till	258.5	79.5	167.0	413.2
Full season	SYN	Till	*230.3*	75.5	101.9	354.4

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A framework for considering coral ecosystem services for biodiversity offsets

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Biodiversity offset practices often focus solely on securing ecological validity, despite biodiversity providing various human benefits such as ecosystem services (ES); the use of which is often lost by both the development project and the offset itself. In this paper, a framework is suggested to rationally examine the compensatory measures for ES use losses and tested with actual offset cases in developing countries, focusing on endangered coral ecosystems. In the framework, we first evaluate the necessity of compensatory measures for the losses of coral ES (CES) uses then suggest the restoration measures of CES uses instead of provisions for livelihood supports. The restoration measures include the provision of alternative sites and improvements to reduce the environmental load of the uses. The framework revealed that the necessity of compensation measures and the suitable restoration measure are varied depending on the original location and type of the CES uses, even within small areas. Together with optimum offset site selection, restriction of the destructive CES uses, integrating existing community-based resource management schemes, these careful considerations of CES in biodiversity offset provides hint that enable local people to achieving a balance between conservation and use. However, state of CES uses and corals should be monitored to ensure the framework effect. We further discuss the condition to apply this framework.

Key words: Biodiversity offset, coral, ecosystem service, restoration, developing country.

INTRODUCTION

Biodiversity offsets (offsetting) are the measurable conservation outcomes from actions taken to compensate for the residual adverse impacts of development projects on biodiversity, after taking prevention and mitigation measures (BBOP, 2012). The goal of offsetting is to reduce the net loss of biodiversity to at least zero, that is, to achieve "no net loss" (Bull et al., 2013; Ledec and Johnson, 2016). However, it is acknowledged that offsetting has various issues, such as the uncertainty of the offset achievement, poor arrangement of long-term monitoring, insufficient evaluation of the offsetting impact on biodiversity value for humans, and the physical

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distance of the prospective beneficiaries of offsetting to the recovery site (Maron et al., 2016; Grimm and Köppel, 2019; Souza et al., 2021). The most fundamental issue of offsetting is the tradeoff between conservation and use; the uses are predominantly supplied by healthy ecosystems well conserved, however, such ecosystem is degraded with the increasing uses (Moreno-Mateos et al., 2015; Sonter et al., 2020). These issues become failure risks of offsetting unless proper considerations are taken.

In developing countries, particularly within rural areas, these issues are more serious. This is because local livelihoods often rely largely on natural resources (Bidaud et al., 2017; BBOP, 2012). However, many offset practices focus only on securing ecological validity (Gelcich et al., 2017). Therefore, international aid organizations have recently requested that the social aspects of offset practices also be considered (BBOP, 2012; Ledec and Johnson, 2016; Jacob et al., 2016). The benefits provided by nature are generally called ecosystem services (ES).

In the long term, offsetting can restore ES loss via ecosystem recovery, if the offset site is located proximally to the impacted area (Agar et al., 2019; Ledec and Johnson, 2016). However, the current offset policy lacks evidence ensuring the offset's potential for ecological restoration (Maron et al., 2012). This is an uncertainty of the offsetting and is caused in part by the poor arrangement of long-term monitoring, which means that the restoration of ES loss is also uncertain (Jossefson et al., 2021). In addition, since a traditional impact assessment lacks explicit guidance, an ES may be qualitatively evaluated as an item such as land use, habitat and land planning, but such evaluations may also miss some ESs (Honrado et al., 2013) and less considered biodiversity value for human beings (Souza et al., 2021). In contrast, during the offsetting period, additional ES losses often occur as natural areas acquired as offset sites and certain uses of the ESs are restricted for offset site management (Ledec and Johnson, 2016; Bidaud et al., 2017). Moreover, ES losses induced by development projects will remain for a certain period until "no net loss" is successfully achieved (Bullock et al., 2011). Due to these circumstances, many local livelihoods could be threatened by ES losses. While, the pattern of ES use varies from site to site depending on demographic dynamics (Honrado et al., 2013). Therefore, such ES losses may not be serious depending on the location.

To supplement the lack of guidance available when considering ES offsetting, ES considerations for the establishment and management of protected areas (PA) could be used as a reference, as both PAs and offset sites ultimately have the same objectives (Benabou, 2014; Bidaud et al., 2017). Furthermore, a number of studies have scientifically and practically demonstrated the key points required for successful PA establishment and management (Edgar et al., 2014; Kelleher, 1999; Lester et al., 2009; Leverington et al., 2010). However, we must pay further attention to the considerations of ES for offsetting because the uses of ES (ES uses), which are affected by both development projects and offsetting, can be concentrated elsewhere, causing conflicts and further resource degradation (Bidaud et al., 2017). Thus, developing a methodology by which to address cumulative ES losses and consequent social problems is a key challenge in offset planning (Jacob et al., 2016).

In recent studies, to tackle the above-mentioned issues, an approach was proposed that selects offset sites using three criteria: (1) those with higher restorability to mitigate uncertainty; (2) those with a lower human dependency on ES to mitigate trade-offs and reduce additional loss and conflict; and (3) those with easy access for users to mitigate the physical distance (Takeda et al., 2020). However, this approach will not solve all offsetting issues; the additional and remaining losses should be supplemented through compensatory measures such as livelihood support or monetary payments, and/or any other suitable approaches.

In conventional practices of development projects and PA management, various livelihood supports have often been provided as compensatory measures against losses or restrictions on ES (Munthali and Mughogho, 1992; Sievanen et al., 2005; Triet, 2010); however, in many cases, the effects of these supports have been questioned or criticized as ineffective (Ireland, 2004; Wright et al., 2015; Roe et al., 2017; Lowe et al., 2019). Furthermore, because of insufficient monitoring data for social changes, many cases have failed to evaluate the effects (Roe et al., 2017; Wicander and Coad, 2018). Given the financial difficulties of developing countries, compensatory measures should be effective. Therefore, the restoration of lost ES uses might be more realistic as a compensation measure.

Coral reefs have developed from the tropics to the subtropics and sustain a rich biodiversity in many countries (Moberg and Rönnbäck, 2003; Yeemin et al., 2006). Most coral reefs are in developing countries within the tropical zone (Birkeland, 1997; Gomez, 1997). However, those coral reefs under threat due to recent development activities and/or urbanization (Cesar et al., 2003; Wilkinson, 2008), as well as rising sea temperatures, and the acidification of seawater (Burke et al., 2001). To address these situations, several coral propagation techniques, such as simple transplantation, transplantation of nursery-raised corals, and electro-stimulation have been implemented on a trial basis (Barton et al., 2015; Jacob et al., 2018). However, fundamental challenges to propagation have also been identified, such as the difficulty in selecting sites that meet physical and biological conditions, the removal of anthropogenic stressors, and the measurement of restoration success and long-term monitoring (Bayraktarov et al., 2016). Furthermore, there are more cautious opinions that state

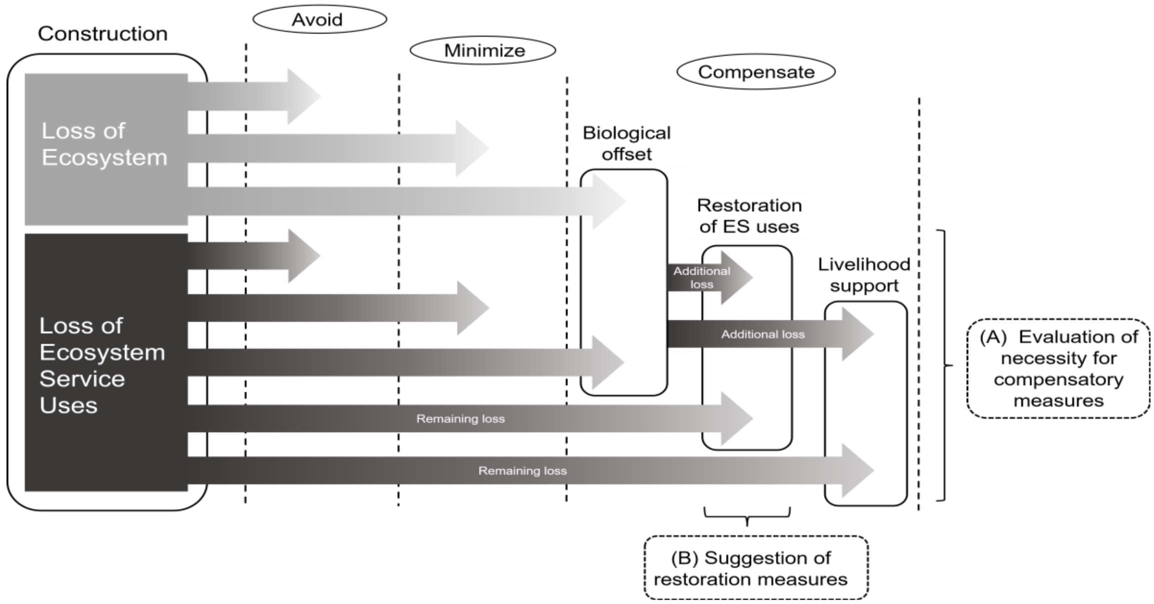

Figure 1. Mitigation hierarchy against losses to ecosystems and ES uses, and study approaches proposed in this paper (indicated by dashed squared circles). The figure is modified those of Takeda et al. (2020).
Source: Authors

there are no established techniques of propagation (Precht et al., 2005; Edwards and Gomez, 2007; Hein et al., 2017). For this reason, offsetting through the establishment and management of PAs is likely to be more realistic as a countermeasure, at least in the mitigation of the adverse impacts of development projects on coral in developing countries. However, a marine environment presents unique challenges such as environmental complexity and difficulty in the governance of resource management, and therefore, a detailed study on how these challenges can be dealt with through an offset practice is expected (Niner et al., 2017, Jacob et al., 2020).

The objective of this paper is to develop a framework that efficiently compensates for the losses of coral ES (CES) uses induced by both development projects and offsetting. The framework rationally evaluates the necessity of compensation and places a priority on restoring the lost CES uses by examining the social situation surrounding ES uses in and around the affected area. the framework was applied to an actual coral offsetting case and discussed its advantages and the possibilities for sustainable CES uses.

METHODOLOGY

Approach

The mitigation hierarchy against the loss of ecosystems and ES uses and the study approaches by which to consider compensatory measures to loss are shown in Figure 1. Since the loss of ES uses may not be serious, the necessity of compensatory measures for each ES use was first evaluated. Once a certain necessity is recognized, restoration measures will be suggested.

Framework

The framework used to examine compensatory measures is described in Figure 2. This framework ultimately aims to consider two items that coincide with the study approach: (A) the evaluation of necessity for compensatory measures; and (B) the suggestion of restoration measures. Item (B) can be further codified into three subcategories; (1) suggestion of measures to improve the CES uses[1] so as not to impact corals; (2) the identification of alternative sites that can accommodate impacted CES uses; and (3) the evaluation of the necessity for livelihood support.

To evaluate the necessity for compensatory measures, we estimated how much each CES use contributes to livelihoods (analysis “1”), how the restriction of CES uses is perceived (analysis “2”), and how much CES use has been impacted by the development project and will be impacted by the offsetting (analysis “3”). Since some ES, such as cultural services, are difficult to replace with a monetary value (Bullock et al., 2011; Calvet et al., 2015; Moreno-Mateos et al., 2015), the contributions of CES uses were estimated using two types of data: The level of original engagement in the CES activities[2] (data “a”) and the purpose of the CES activities (data “b”). The perception to the restriction is

[1]CES uses refer to local people’s uses of provisioning and cultural services of coral ecosystems that are restorable through offsetting.
[2]CES activities refer to the specific social activities using the provisioning and cultural services of the coral ecosystem.

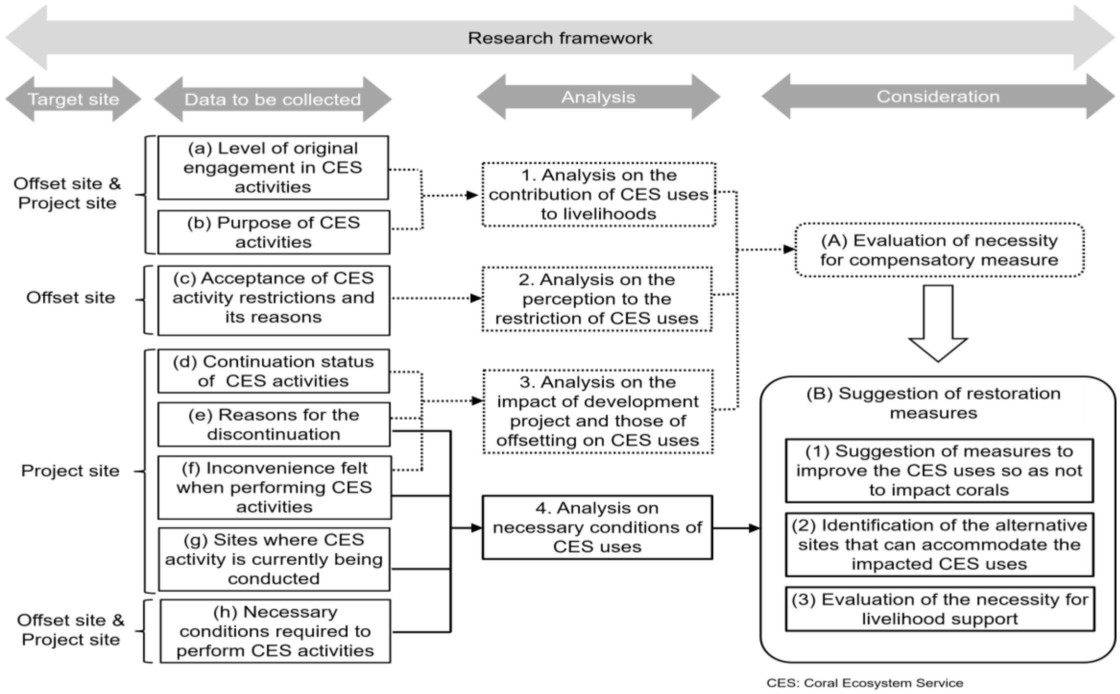

Figure 2. Framework for considering compensatory measures.
Source: Authors

estimated using direct data about the acceptance of the restrictions and its reasons (data "c"). The impacts of the development project and offsetting on CES uses are estimated from the CES activities continuation status (data "d"), reasons for the discontinuation (data "e"), and the inconvenience felt when performing the CES activities (data "f").

If specific CES uses have a high need for compensation, the content of the restoration measures is examined for those uses. To examine these, the necessary conditions for CES uses (analysis "4") are estimated from four types of data: Reasons for the discontinuation (data "e"), inconvenience felt when performing CES activities (data "f"), sites for the current CES activities (data "g"), and necessary conditions required to perform the CES activities (data "h").

Eventually, the necessity for compensation measures and the restoration measures are comprehensively examined for each CES activity and original site uses. If reasonable restoration measures are not found, the necessity of the livelihood supports is evaluated.

Application to a coral offset case

This framework was applied to a coral offsetting case induced by a wharf development project in the Republic of Vanuatu. The idea of offsetting in this case is that offset coral losses by creating a new PA at a site where mechanically damaged corals exist in a Community Conservation Area (CCA) that is under registration process. The outputs of the framework are expected to be incorporated into the CCA management plan, and the restriction of activities will be newly set, specifically for the offsetting on top of the CCA regulations, to secure the additionality of the offsetting. Two (2) offset sites, named Ifira East and Fatumaru in Port Vila Bay (where the wharf project site is located), were officially selected as the offset sites from four candidate sites (Figure 3) after analysis of their advantages and disadvantages (Takeda et al., 2020). Those advantages and disadvantages were evaluated based on the following: (1) levels of dependency on CES, (2) restorability[3], and (3) accessibility for CES users and/or managers. Ifira East is advantageous because of its high restorability, while Fatumaru is advantageous in terms of both its restorability and lower dependency. These offset sites were declared by the executing agency to the residents of Port Vila City after a series of consultations with stakeholders, including the primary CES users and the resource managers of Port Vila Bay.

Surveys

A preliminary survey was conducted in October 2017 before construction of wharf was completed, in which random interviews were held on the street around the local market, schools, and residential areas of Por-Vila City. The aim was to test the question items to be used for the main survey and to roughly grasp the CES activities performed around Port Vila Bay. This interview style was adopted as the required information did not depend on the locality, and with the aim to hear opinions directly from various people. Response alternatives for questions were predetermined based on the census report of Port Vila City (McEvoy et al., 2016) and advice from the Japan International Cooperation Agency local office. A total of 11 residents responded to the interview.

The main survey was conducted in January and March 2018 after construction was completed, and it used face-to-face structured

[3] The term "restorability" refers the possibility of restoring ESs through ecosystem restoration.

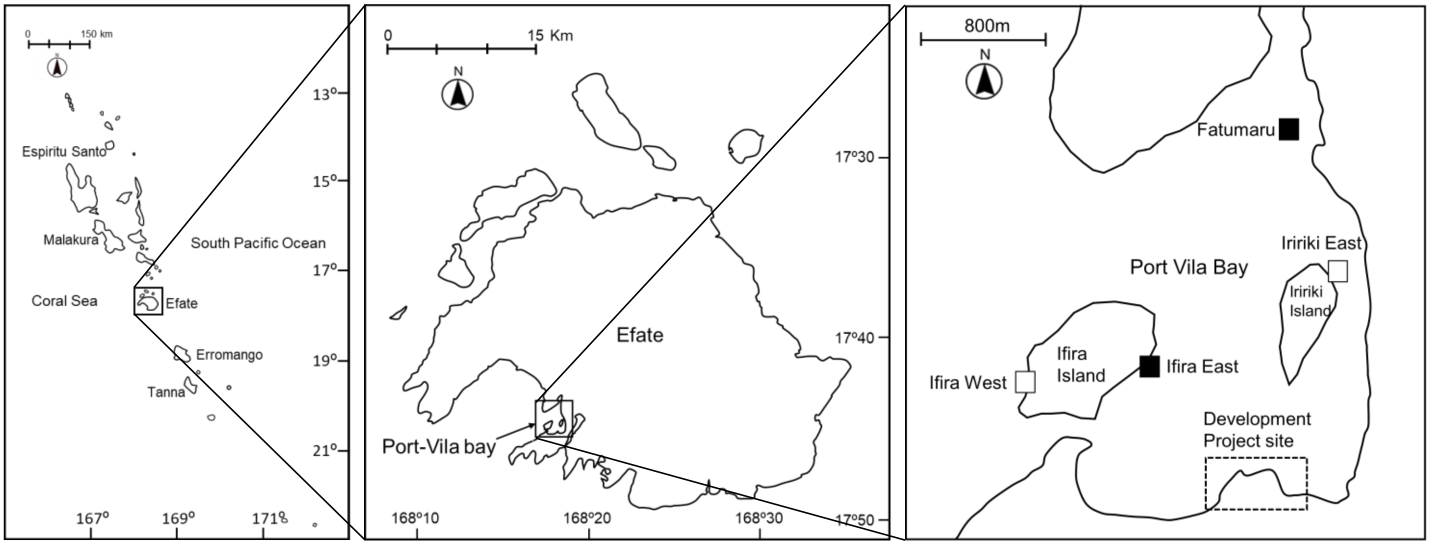

Figure 3. Location of Port-Vila Bay and the offset candidate sites (rectangle) and offset sites (filled rectangle) selected therein.
Source: Authors

interviews with CES users of the wharf development project site (development project site) and offset sites (Ifira East and Fatumaru) to collect data “a,” and “b,” as described previously. Additionally, between December 2018 and July 2019, further face-to-face structured interviews were conducted with CES users to collect data “c,” “d,” “e,” “f,” “g,” and “h,” also as described previously. An interview style was used rather than questionnaire surveys, after considering the difficulty of collecting filled-in questionnaire sheets from the respondents due to cultural background and the social circumstances of Vanuatu. Preliminary surveys found that most of the locals used the provisioning and cultural CES in some manner around Port Vila Bay, and that most locals could thus be considered CES users. Therefore, in the main survey, the CES users targeted for the interviews were not specifically pre-identified. Instead, the location where the main CES users of each site live were pre-identified with advice from the executing agency and a census report of Port Vila City (McEvoy et al., 2016). Furthermore, instead of a random sample taken from the entire population of the target site, the locals who were willing to communicate on the interview date and time were interviewed. Similarly, as the study did not employ a purely quantitative approach to prove a hypothesis but adopted a mixed method that combines qualitative and quantitative examination, the sample size was not defined. All interviews were conducted individually without prior notice to avoid response bias resulting from communication among interviewees. The interviewees were Vanuatuans between age of 11 and 79; gender, affiliation, religion, and tribe were not considered. The details of the interviews are presented in Table 1. The number of responses used for the analysis was less than the actual number of interviewees because no answers were obtained from all interviewees depending on the analysis types.

The questions and answer options used in the main survey are described in Table 2. For most questions, choices were given considering the ease of answering and to avoid unit misunderstandings. Since the preliminary survey identified four CES activities, mainly performed in Port Vila Bay: Fishing, recreation, tourism business, and sand mining, the interviewees were asked about these CES activities in all questions. For the level of engagement in CES activity, the interviewee was asked not only for their original engagement, but also its frequency and duration, as these contribute to our understanding of their dependence on the CES. This assumption, for example, is supported by Salagrama (2006) who emphasizes a clear correlation between the number of working days of fishermen and their food security. For the purpose of the CES activities, recreation and tourism business were clearly conducted for spiritual fulfillment and income generation, respectively. Therefore, data were collected only for fishing and sand mining. In general, sustainable livelihoods could not be achieved from securing food alone, but a cash income was also required. Therefore, if “for selling” was frequently answered, these activities are more likely to contribute to the interviewees’ livelihood. On acceptance of the activity restrictions and continuation status of the CES activities, the reasons were asked but interviewees were not forced to give their answer to avoid disruptions to the social order.

For the necessary conditions required to perform CES activities, the answer options and categories provided are described in Table 3. Since the options provided may be insufficient, "others" was also available to allow a free answer. Among the categories, “substance” reflects the substantive characteristics of provisioning and cultural CES. Therefore, if “substance” is frequently answered, similarities in CES should be considered when selecting alternative sites.

Data processing and analysis

The percentage of each answer was calculated by dividing the number of answers by the total number of answers for each site and/or for each CES activity; except for the reason for the discontinuation as not enough answers were obtained and the inconvenience felt which requires an answer regardless of the site and CES activity. For the level of original engagement in the CES activities, the engagement rate, weighted sum of engagement frequency, and engagement duration were multiplied with each other for each activity to optimize the engagement level. For the inconvenience felt, the frequency of answered option within a category was first divided by the number of answer options, then the percentages of each category were calculated in addition to the

Table 1. Details of the interviews of main survey.

Interview	Interview timing	Target site		Interview location	Number of the interviewee	Gender ratio of the interviewee (Male/Female)	Demography				
							Children (0-14 years old) (%)	Youth population (15-29 years old) (%)	Population aged (30-59 years old) (%)	Older population (60-100 years old) (%)	Unknown (%)
1st Interview	Jan. and Mar. 2018	Offset sites	Ifira East	Ifira Island	90	1.6	2.2	50.0	38.9	8.9	0.0
			Fatumaru	Anabrou-Melcoffee Ward	100	1.6	0.0	57.0	38.0	5.0	0.0
		Development project site		Ifira Island	91	1.8	2.2	33.0	52.7	9.9	2.2
2nd Interview	Between Dec. 2018 and Jan. 2019	Offset sites	Ifira East Fatumaru	Ifira Island and Port-Vila city	50	2.3	0.0	37.2	48.8	9.3	4.7
		Development project site		Ifira Island	43	4.4	0.0	22.0	72.0	2.0	4.0
Reference		Port-Vila & Ifira*			-	1.0	31.2	32.3	32.4	4.1	0.0

*Data cited in Vanuatu National Statistics Office (2016).

calculation of each answer percentage. The percentage of each category was organized for the development project site and for offset sites to propose suitable restoration measures for each of these site categories. Since statistical analysis using a mixture of qualitative and quantitative data is not realistic, each data category was individually organized and the analysis results were comprehensively interpreted with respect to the previously described procedure for a qualitative study introduced by Otani (2017).

RESULTS

Contributions of the CES uses to livelihoods

The level of original engagement in the CES activities at each site is shown in Figure 4. Fishing consistently showed high engagement levels among the sites, indicating that fishing is a major activity in Port Vila Bay. While there was also a high level of engagement for recreation, its activity levels fluctuated between the sites. For tourism business, engagement was high at Ifira East and the development project site, but extremely low at Fatumaru, while sand mining showed the lowest engagement levels across all sites. Among the CES activities, it is obvious that tourism business is a source of income and that it contributes to people's livelihood. However, it is uncertain immediately whether fishing contributes to people's livelihood as they may also fish for recreational purposes. However, recreation plays an important role in the spiritual fulfillment of people (Millennium Ecosystem Assessment, 2003), and therefore, fishing might indirectly contribute to their livelihood. Sand mining is highly likely to contribute to livelihoods as a source of income and as a construction material.

The purposes given for the CES activities are summarized in Figure 5. For fishing, "for self-consumption" is the most prominent purposes while "for selling" was less answered among the sites. However, if including the combined option of "both," the portion of "for selling" increased at Ifira East and the development project site; therefore, fishing is a main means of livelihood for certain people and at certain sites. For sand mining, more than 90% of the responses were "for personal use," although the number of responses was low. These results combined with the low level of engagement in sand mining, indicate that it is not a critical CES activity that sustains people's livelihood.

Perception to the restriction of CES uses

The acceptance to the restrictions of CES activities is presented in Figure 6. For fishing, "totally accept" was the highest (62%), however, "partly/conditionally accept" was low (6%); therefore, its total acceptance percentage was less than 70%. In contrast, for recreation and tourism businesses, there were fewer "totally accept" responses (34 and 42%, respectively), but a higher percentage for "partly/conditionally accept" (38 and 32%, respectively); consequently, its total acceptance percentage was more than 70% for

Table 2. Question and answer options in the main survey.

Data to be collected	Question	Answer option or unit
Level of original engagement in CES activities	Had you been doing any activities in the sea area?	Fishing / recreation / tourism business / sand mining*
	How often had you been doing it?	Days / month
	How long had you been doing it?	<1 year / 1-5 years / 6-10 years / 11-15 years / 16-20 years / >20 years
Purpose of the CES activities (only for fishing and sand mining)	What was the purpose of those activities?	For self-consumption (For personal use) / For selling/ both
Occupations of the engaged persons	What is your occupation?	Fishermen / Farmers / Company employees / Shop employees / Shop owners / Police / Military service members / Other occupations / No occupation*
Acceptance of activity restrictions and its reasons	Do you accept activity restrictions for offset site management?	Totally accept / Partly or conditionally accept / Barely accept / Fully reject
	What is the reason for acceptance/rejection?	Free answer
Continuation status of CES activities	Are you still doing those activities?	Continued / discontinued
Reasons for the discontinuation	What is the reason for a discontinuation?	Free answer
Inconvenience felt when performing CES activities	Do you feel inconvenience to do the activities?	Yes / No
	If you feel please specify the reason.	Free answer
Sites where CES activity is currently being conducted	What is the site where activities are currently being conducted?	Star wharf (development site) / Ifira east coast (offset site) / Iririki east coast (offset candidate site / Vatumaru bay (offset site) / others*
Necessary condition to perform CES activities	What is the necessary condition for performing the activities?	See Table 3*

*Multiple answering is allowed.
Source: Authors

each activity. Meanwhile, sand mining obtained moderate “totally accept” (48%) and low “partly/conditionally accept” (12%) responses; consequently, its total acceptance percentage was the lowest at 60%.

The reasons for acceptance are listed in Table 4. The main reason for full acceptance is concerning the negative environmental impacts of each activity. This means that CES users were interested in resource management. In contrast, the most common reasons for partial or conditional acceptance were to segregate restricted and non-restricted areas, depending on the destructiveness of the activities. According to Takeda et al. (2020), most recreational activities and tourism businesses do not have positive correlations with the mechanical damage of corals, but they do have the potential to cause damage in shallow areas. Thus, if the activities to be restricted could be rationally identified considering the environmental load and if the restricted and non-restricted areas could be segregated as zoning, compensatory measures may not be required for all activities.

Impacts of development projects and offsets on CES uses

The continuation status of CES activities is

Table 3. Necessary conditions required to perform CES activities provided in the main interview.

Category	Options given to interviewees for their answering			
	Fishing	**Recreational activities**	**Tourism business**	**Sand mining**
Substance	Many fish	Beautiful place Good atmosphere High safety	Beautiful environment Many tourists	Sand Quality of sand
Resources	Available fishing gear Co-workers	Clean place Less crowded Corals Many fish	Many fish Corals Co-workers	Co-workers
Access	Easy access Close to fish landing site Close to fish market	Easy access	Easy access Close to tourism agent	Easy access Close to residence Close to market
Rules	Legally/customarily allowed	Legally/customarily allowed	Legally/customarily allowed	Legally/customarily allowed
Others	Others (Free answer)	Others (Free answer)	Others (Free answer)	Others (Free answer)

Source: Authors

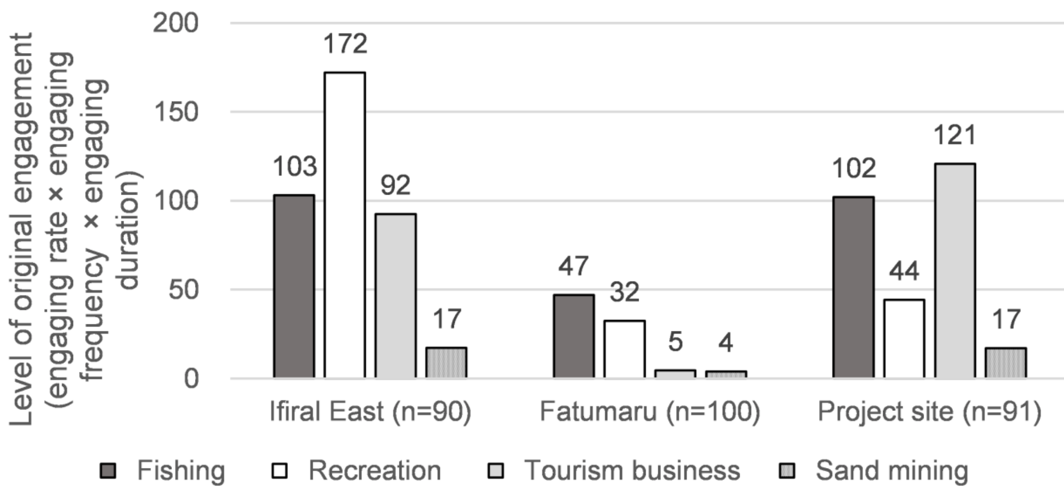

Figure 4. Level of original engagement in CES activities by site.
Source: Authors

described in Figure 7. The highest continuation occurred in sand mining (100%), followed by fisheries (71%), tourism business (63%), and then recreation (57%). Since sand mining exhibited a low engagement level and had a low number of respondents, this result may not be plausible. These results infer that certain CES users discontinued their relevant activities. However adverse impacts from the development project and offsetting on the CES uses cannot be predicted from this data alone. Furthermore, only four respondents provided answers for the reasons for discontinuation. One responded that pollution had increased, and the remaining three responded that the development project itself was the reason. It is difficult to hypothesize why pollution increased, but the responses suggest that the development project impacted the continuity of the CES activities to a certain extent.

The inconvenience felt when performing CES activities is shown in Figure 8, and the number of respondents who experienced an inconvenience (33%) was greater than those who did not (21%). Pollution, resource degradation, and marine waste were the major inconveniences felt, as

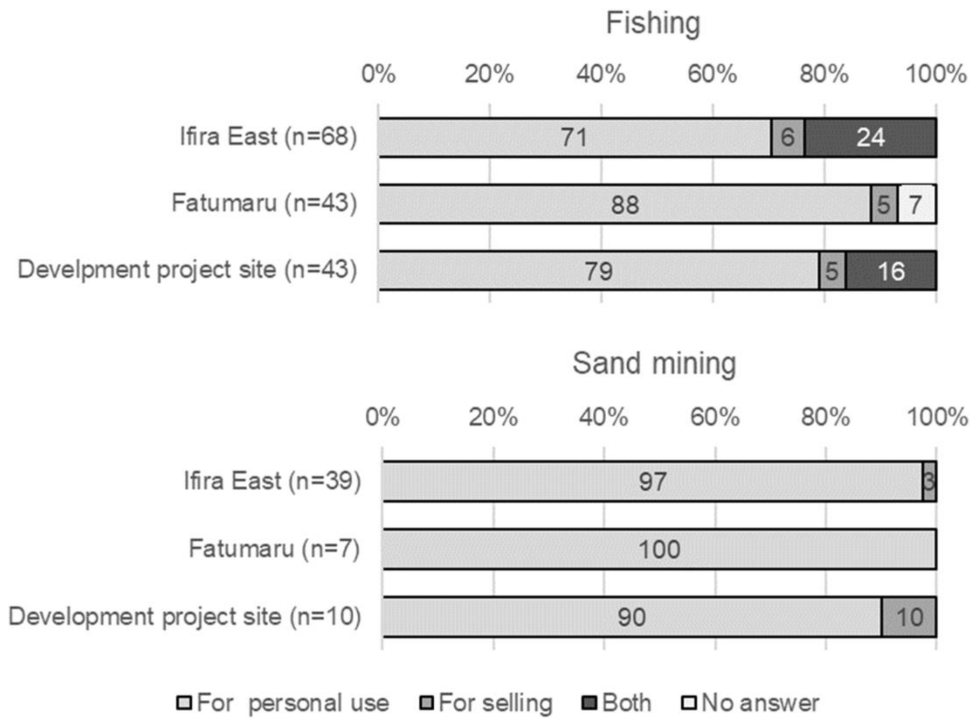

Figure 5. Purposes identified by respondents for engaging in CES activities.
Source: Authors

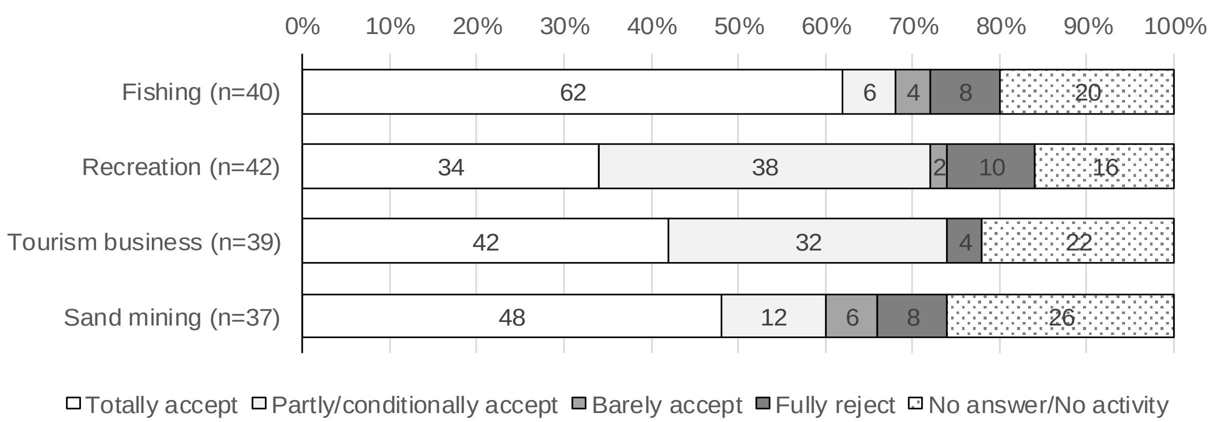

Figure 6. Acceptance to restrictions on activities for offset site management.
Source: Authors

shown in Figure 9. However, other respondents also cited unwelcome rules and regulations and fuel use increases as inconveniences.

These results do not clearly indicate the impact of the development project but suggest that the impacts from construction and new wharf operations may increase environmental degradation and force the original CES uses to move to other areas. The forced movement can also be caused by offsets, suggesting the offset impact on CES uses. However, the degree of offset impact may be less than the development project impact as the offset theoretically does not cause environmental degradation.

Necessary conditions of the CES activities

The sites that responded, where CES activities are currently being conducted, are summarized in Table 5.

Table 4. Reasons for accepting restrictions.

Reason	CES activities			
	Fishing	Recreation	Tourism business	Sand mining
Reasons to totally accept the restrictions	・Restrictions are necessary for fish and marine organism reproduction ・To create a beautiful environment for marine life and human beings ・Marine resources can be recovered following the restrictions ・Restrictions are a necessary practice since marine resources are decreasing ・Resource management and avoiding over-exploitation ・Restrictions ultimately result in higher incomes ・Fishing impacts the environment and marine resources ・We have to follow the regulation ・Fish populations are decreasing ・To conserve the environment ・To sustain marine life	・People can continue recreational activities in another place ・To create a beautiful environment for marine life ・Recreation impacts on resource and environment ・To avoid environmental disturbances ・People destroying marine resources ・To pursue the miracle of Efate Island ・To conserve the environment ・To protect the environment ・To conserve corals	・Tourism can provide livelihoods but impacts the environment and marine resources ・Restrictions are necessary to avoid environment disturbance ・Tourism leads to disturbances of the marine environment ・Tourism impacts the environment and marine resources ・Beach usage should be altered for children ・To help tourists create a better environment ・Restrictions in limited areas are not a problem ・Tourism destroys marine resources ・Tourism destroys the environment ・Tourists walk on corals	・To mitigate soil erosion and associated sea level rise, which is a common issue in the Pacific Islands ・Sand is decreasing and erosion seems to be progressing ・Sand mining impacts the environment and marine resources ・Sand mining induces coastal erosion ・To avoid damage caused by a sea level increase ・Sand amount is decreasing ・To recover marine life
Reasons to partly/conditionally accept the restrictions	・If fishing methods that do not damage corals (e.g. shore fishing, boat fishing, offshore fishing) are allowed ・If restricted area and non-restricted areas are separated	・If non-destructive activities are allowed (because swimming and snorkeling are a part of life). ・Restrictions should be dependent on the type of recreational activity If restricted area and non-restricted areas are separated ・Small areas should be secured to continue recreational activities ・If alternative places are prepared	・The livelihoods of some people depend heavily on the tourism business (these people should be excepted) ・If restricted area and non-restricted areas are separated	・Collections of small amounts of sand should be allowed
Reason for barely accept the restrictions	・No answer	・No answer	・No answer	・The amount of sand cannot be recovered through coral offsets
Reason for fully reject the restrictions	・No answer	・Recreation is not harmful	・No answer	・No answer

Source: Authors

The sites with the most respondents were the development project site (33%), followed by Ifira West (23%), and Ifira East (19%). Ifira East is the closest to the development project site followed by Ifira West. These results indicate that CES users tended to continue their activities at the original

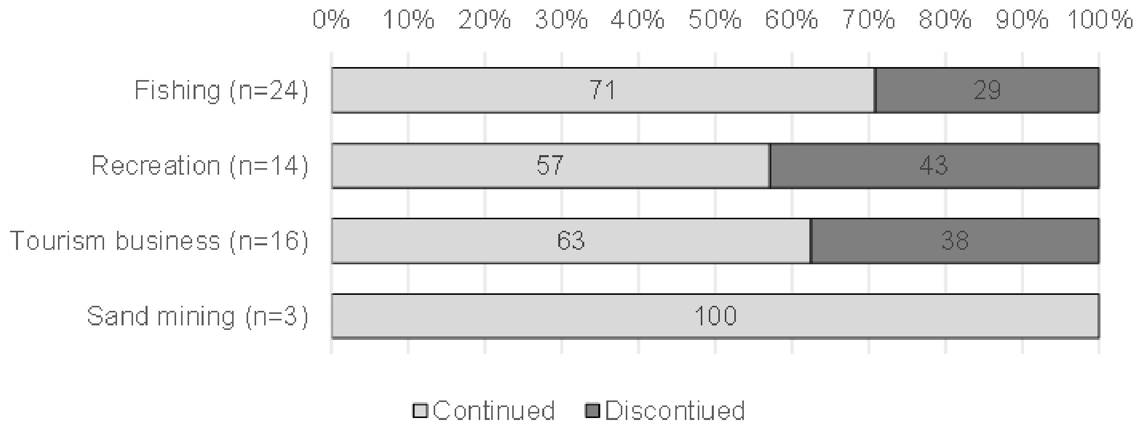

Figure 7. Continuation status of each activity.
Source: Authors

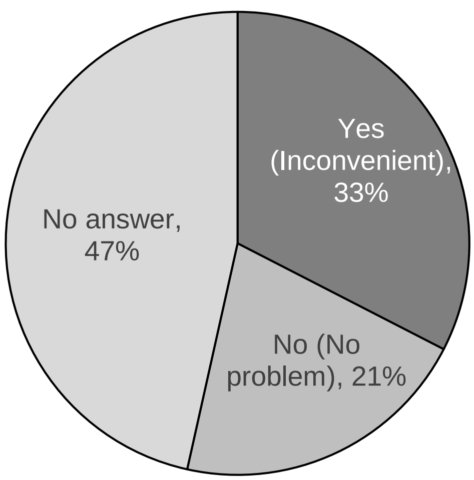

Figure 8. Inconvenience felt when performing CES activities (n = 43).
Source: Authors

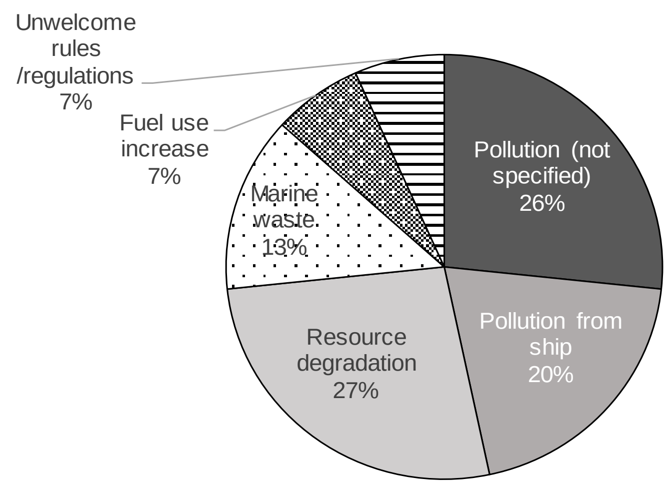

Figure 9. Types of inconvenience (free description, n = 13).
Source: Authors

location or within its vicinity after construction completion. Notably, recreation and sand mining continued mainly in Ifira West but not at the development project site. In contrast, fishing and tourism businesses continued mainly at and near the development project site, thereby suggesting that fishing and tourism businesses could be continued relatively easily, even during new wharf operations. These results imply that good access could be an important condition for certain CES activities.

Considering the reason for discontinuation and the inconveniences felt, healthy environments are supposedly one of the most important conditions for CES activities. However, since fishing and tourism businesses could continue around the development project site where the environment was degrading, there may be more important conditions to consider, depending on the CES activities.

The responses to the necessary conditions and their categories are illustrated in Figures 10 and 11, respectively. It is obvious that many responses belong to the substantive condition that reflects the CES (Figure 11). This means that many CES users are aware of the substantial characteristics of the CES when performing their activities; for example, “Many fish” was the most frequent answer for fishing. Consequently, an area that accommodates many fishes should be selected as an alternative site for fishing. Furthermore, for recreation in the offset sites, “easy access” would be required, based on the responses. This is possibly because recreation often requires frequent traveling. Additionally, rules are also a necessary condition especially for recreation and sand mining. This may explain why recreation and sand

Table 5. Sites where CES activities are currently being conducted (multiple answers, n = 21).

Activity	Development project site	Ifira East	Ifira West	Iririki East	Fatumaru	Others (inc. not specified)	Total
Fishing	9	6	6	5	1	2	29
Recreation	3	1	5	1	0	2	12
Tourism business	7	5	2	5	0	0	19
Sand mining	1	0	2	0	0	0	3
Others	1	0	0	0	0	0	1
Total	21	12	15	11	1	4	64
Percentage	33	19	23	17	2	6	100

Source: Authors

mining mainly continued outside of the development project site (Table 4), specifically because of new rules formulated for wharf operations.

Consideration of restoration measures

The necessity of compensation and the restoration measures are shown in Table 6. Fishing is a prominent CES activity among the sites. Higher dependence on the living marine resources and higher engagement in fishing around Port Vila City was also previously reported by Trundle and McEvoy (2015). As reasons for the discontinuation and the inconvenience, environmental degradation supposedly induced by the development project was often the response. These responses indicate that the necessity of compensation is high. However, fishing is not a major means of income generation. Additionally, the interviewees tended to accept fishing restrictions to enable resource management. From these, the necessity of compensation can be evaluated as moderated for the development project site. While the necessity is low for the offset sites because offsetting theologically does not cause environmental degradation and an increase in the abundance of fish can be expected due to the offset's conservation effect. The most important condition for fishing is fish abundance, and the current major fishing sites are the development project site and Ifira West, which suggests that fish are abundant at these sites. Therefore, the following restoration measures were suggested: (1) An alternative fishing site should be designated at the development project site but rules for safety and the prevention of marine pollution should also be installed; (2) An alternative fishing ground should also be designated at Ifira West, but measures such as the installation of a floating pier and an information board citing the environmental rules are desirable to avoid further coral damage.

The level of engagement in recreation was high at Ifira East and Fatumaru. However, a certain number of interviewees discontinued the recreation and/or found inconveniences to enjoy them, after the construction was completed. Furthermore, many interviewees suggested restriction/no restriction zoning for specific recreational activities as a condition of the recreation restrictions. In general, recreation keeps people mentally healthy. Considering these points, the necessity of compensation is high for Ifira East and Fatumaru users. For the restoration measure option, good access was found to be a key. Therefore, alternative sites should be designated in the vicinity of the original sites. To avoid further mechanical damage to the corals via trampling, alternative sites should be located at a sandy beach or should have a certain depth. Some sandy areas with patchy coral colonies between Ifira Island and the development project site, as well as the adjacent coastal area of Fatumaru, are appropriate as they are both located proximally to the original sites. To ensure that further coral damage is avoided measures should be taken, such as installing a floating pier and an information board to cite the environmental rules.

Tourism businesses contribute to people's livelihood, and engagement in them was high at Ifira East and development project site. In addition, a certain number of interviewees reported that they discontinued their businesses and/or experienced inconvenience to perform their business due to the development projects. Furthermore, many interviewees suggested restriction/no restriction zoning for specific tourism as a condition of the tourism restrictions. Considering these points, the necessity of compensation is high for Ifira East and project sites users. The most frequently identified condition for a tourism business was "a beautiful environment", followed by "many tourists." Moreover, the development project site was the most frequently identified location for current tourism businesses, followed by Ifira East and Iririki East. This infers that local people gather tourists at the new wharf and take them to surrounding sites to let them enjoy the beautiful landscape. The demand for tourism business will increase

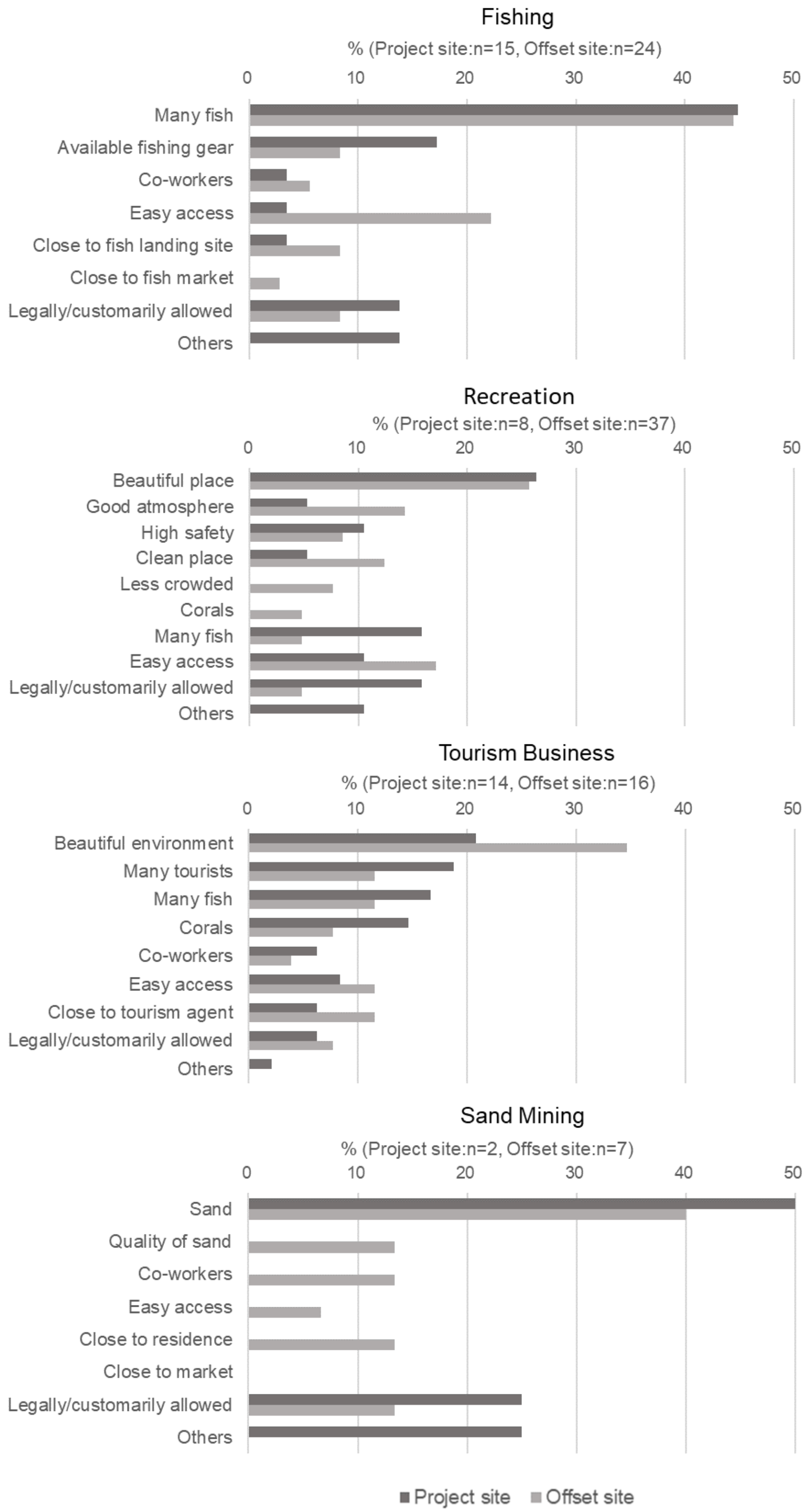

Figure 10. Responses identifying the necessary conditions to perform CES activities.
Source: Authors

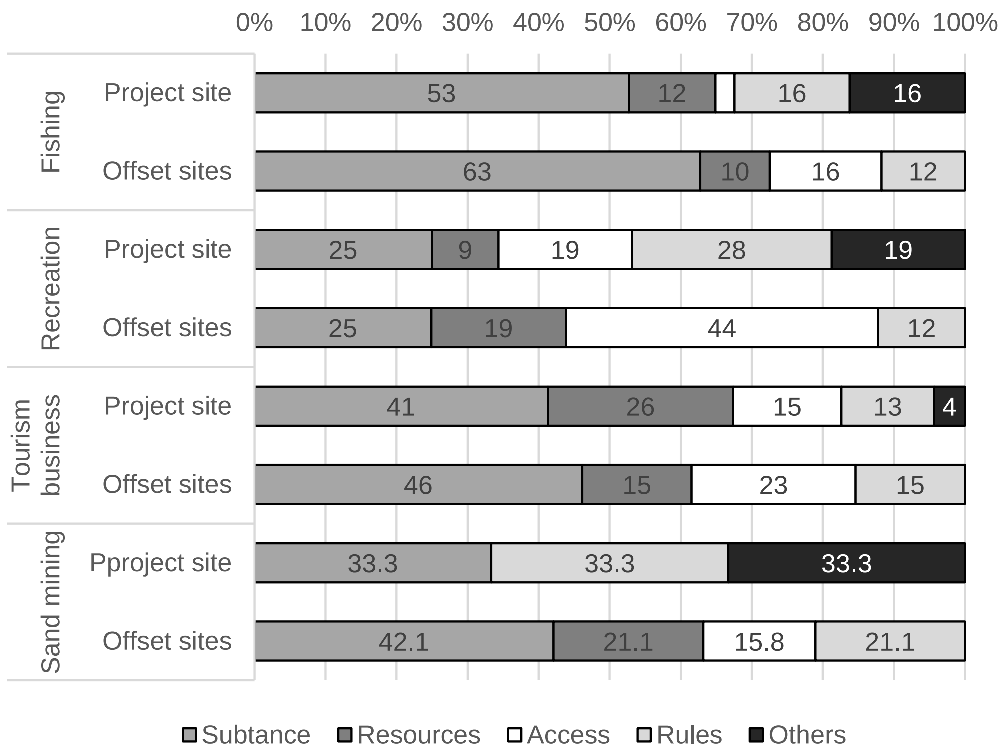

Figure 11. Categorized responses for the necessary conditions to perform CES.
Source: Authors

Table 6. Summary of the proposed restoration measures.

Activity	Necessity of compensatory measures	Alternative site that can accommodate CES activities	Measures to improve CES activities so as not to impact the corals	Necessity of livelihood supports
Fishing	Moderate for development project site users; Low for Ifira East and Fatumaru users	Development project site Ifira West	Set-up of rules for safety fishing and prevention of marine pollution at development project site; Installation of floating pier on shallow reefs and information board that cites the local environmental rules	Low so far
Recreation	High for Ifira East users and Fatumaru users; Moderate for development project site users	Area between development project site and Ifira Island Vicinity of Fatumaru	Installation of floating pier on shallow reefs and information board that cites the local environmental rules	Low so far
Tourism business	High for Ifira East users and development project site users; Low for Fatumaru users	Development project site Iririki west coast	Installation of floating pier on shallow reefs and information board that cites the local environmental rules	Low so far
Sand mining	Low for all sites	No need to specify	As needed basis	Low so far

Source: Authors

with the operation of the new wharf, and consequently, the development project site should be designated as an alternative site for gathering tourists. Instead of Ifira East (an offset site), Iririki West which has beautiful sandy beaches should be suggested as the alternative site for tourists. However, tourism has the potential of causing coral degradation. Therefore, the installation of a floating pier and the establishment of rules such as ban on standing and trampling on the corals should be undertaken.

For sand mining, the level of original engagement was low at all sites, and the purpose was personal use, and thus it is not considered a major means of livelihood. Additionally, many of the CES users continued their sand mining after construction was completed, and the acceptance level for sand mining restrictions is generally high. Considering this information, compensatory measures are not immediately required and should be adopted on an as-needed basis.

Thus, a combination of alternative sites and improvement measures could be suggested for all CES activities except for sand mining, which has low compensation necessity. Overall, the necessity of livelihood supports was low for all CES activities.

DISCUSSION

Advantages and disadvantages of the framework

Offsetting could have potential conservation effects. In addition, through the continuation of CES activities at multiple alternative sites with minimum inconvenience and reduced environmental load, it may be possible to minimize the concentration of CES uses and consequent conflict, and eventually balancing between conservation and use theoretically can be achieved. However, to confirm such outcomes for the framework, time series change for the CES uses and the coral state should be monitored for certain periods after the enforcement of relevant laws and regulations. Of course, the cooperation of stakeholders is essential. In this context, the framework is incomplete. The framework only proposed reasonable alternative sites and improvements of the CES uses, and did not confirm the adequacy of those measures.

In Port Vila Bay, Takeda et al. (2020) previously found a positive correlation between some CES uses and coral damage. For example, line fishing and spear fishing were correlated with mechanical coral damage, which may be caused by walking and/or standing in a shallow area (Takeda et al., 2020). These fishing methods are common in some communities around Port Vila Bay (McEvoy et al., 2016). In this context, measures proposed through the framework, such as the dispersion of alternative sites, awareness creation, and the installation of floating piers, could help to avoid further coral degradation while maintaining CES uses, and are therefore advantageous. The framework revealed that the social situation and changes necessary to propose compensation measures, such as contribution of the CES uses to livelihoods, perceptions of the CES use restrictions, conditions of CES uses and, actual adverse impacts of the development project and offsetting, all varied from site to site, even within a relatively small area. This helped to screen and prioritize the CES uses that require compensation and consequently enabled us to suggest alternative sites and improvements for CES uses. However, donor's safeguard policies also stipulate the consideration of social situations and changes through baseline socioeconomic studies and consultations with locals, to ensure the project's risks (e.g., The World Bank, 2016). This means that this idea of the framework is not new.

The framework is unique in that the CES, which are difficult to quantify, are rationally considered for both the development project and offsetting and it can suggest restoration measures per CES uses by considering the characteristics of the original location and use style. For example, the framework indicated that fishing at the development project site was impacted by environmental degradation but continued after construction completion. In contrast, fishing at an offset site may not be largely impacted by degradation. Eventually, the continuation of fishing at the development project site while setting rules for fishing safety and for the prevention of marine pollution was proposed, having found that the development project site has a higher abundance of fish.

It is notable that the applied project was in the implementation stage and this situation made it possible to evaluate the actual impacts; some locals were forced to use CESs elsewhere, felt they were inconvenient to use, or even forced to stop their use altogether. Such implementation stage cases may still fail to evaluate the actual impacts unless monitoring data is available. This means that the framework can be conditionally used.

A way forward for the sustainable use of coral ecosystems

In the same offset case for Vanuatu, the offset sites that have a high restorability, lower human dependency on CES, and good accessibility were officially selected, and the CES activities to be restricted at the offset sites were announced before this investigation to mitigate the social impacts of development and offsetting. However, these measures lacked solutions to manage further social issues, that is, the remaining losses and additional losses of CES uses had to be addressed. To respond to this issue, this investigation has identified suitable alternative sites and improvements of CES uses as a compensation measure.

To manage PAs effectively and functionally, management regimes should respond to the goals of local communities (McClanahan, 2006). For Vanuatu, the offset sites will be internalized within the CCA that the local community is trying to establish for the purpose of resource management. Although this localized situation is not applicable for all offsetting cases, aligning offsetting with community-based resource management activities is nonetheless beneficial. Thus, by using optimum offset site selection, restrictions of the destructive CES uses, provision of alternative sites for the uses, and the improvement of the uses and integrating existing community-based resource management schemes, offsetting became a more realistic strategy by which to achieve the sustainable use of endangered coral ecosystems. However, to ensure the function of the framework, the social and environmental changes should be continuously monitored in a participatory manner respecting community initiatives and with financial and technical support of relevant authorities after taking these measures in the offset case of Vanuatu.

Conclusion

Although development projects can benefit nations, the deterioration of ecosystems associated with these projects can negatively impact local livelihoods. This paper suggested a framework by which to examine how these social impacts can efficiently be compensated in offsetting practices. By applying the framework to an actual coral offsetting case, it was found that there is not always a high necessity level for compensation and those suitable alternative sites to restore CES activities can differ depending on the original location of the CES activities. Furthermore, the framework enabled us to propose improvements of the CES use to reduce environmental degradation while maintaining the benefits of the use at alternative sites. Even though offsetting itself has the potential to conserve degraded ecosystems, it was also expected that these findings will facilitate the sustainable uses of coral ecosystems, which are increasingly threatened. However, to ensure the function of this framework further social and coral monitoring are required.

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CONFLICT OF INTERESTS

No potential conflict of interest was reported by the authors. Though the study cited throughout this work was planned as part of PhD studies undertaken by the lead author, the interviews were conducted by the Japan International Cooperation Agency (JICA), a donor agency of the wharf development project. Therefore, all data shown in this paper were provided by JICA. The views expressed in this paper, however, are those of the authors alone and do not reflect the official view of JICA. The author did not receive any specific grants from funding agencies in the public, commercial, or not-for-profit sectors.

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Highlighting the diversity of the rhizosphere mycobiome of five native West African trees

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Soil microbial communities play a vital role in ecosystem functioning by enhancing mineral nutrition and protecting forest trees against pathogens through mycorrhizal symbiosis. However, knowledge of the diversity and assemblage of belowground fungal communities associated with native host trees in tropical Africa is incomplete. Using high-throughput sequencing, this study examined soil fungal communities in the rhizosphere of five ectomycorrhizal trees (EcM) from (5) countries using ITS and LSU regions. Unconstrained ordination of fungal species was performed using principal component analysis based on their EcM tree rhizosphere affiliation. The ANOSIM test assessed the similarity between the fungal community composition associated with the EcM trees. Overall, 90 species belonging to 84 genera, 71 families, 40 orders and 4 phyla were identified. Soil fungal communities were host specific (P = 0.001). Basidiomycota were more frequently observed in the rhizosphere of Fabaceae, except for *I. doka*, whereas Ascomycota are more abundant in the rhizosphere of Phyllanthaceae (*U. togoensis*) and Dipterocarpaceae (*M. kerstingii*). The genus *Sebacina* is predominantly linked to *M. kerstingii* and *I. tomentosa*, while *Russula* is dominant under *B. grandiflora* and, *Inocybe* with *I. tomentosa*. This study provides new insights into in the rhizosphere of native forest trees in West Africa and highlights areas for future research.

Key words: DNA metabarcoding, ectomycorrhizal association, molecular species, Soil microorganisms, soil fungi, timber trees.

INTRODUCTION

The rhizosphere is considered to be the narrow zone of soil immediately surrounding plant roots (Marschner et al., 2004; Olahan et al., 2016). This area is home to a wide range of interactions between plant roots and microorganisms, which affect soil physical, chemical, and biological processes that sustain biodiversity and ecosystems (Nihorimbere et al., 2011; Sathya et al., 2016; Lu et al., 2018). A major group of microorganisms found in the rhizosphere are fungi, responsible in part for colonizing the roots of a plethora of plant species

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(Olahan et al., 2016; Sathya et al., 2016; Dlamini et al., 2022). Rhizospheric fungi play a vital role in the soil food chain, participating in the recycling of soil carbon and nutrients (Larekeng et al., 2019; Pattnaik and Busi, 2019), and the transformation of hard-to-digest organic matter (such as lignin and other soil organic matter) into usable forms for other organisms (Stokland et al., 2012; Grzyb et al., 2021). Through enzymatic activities, fungal hyphae physically bind soil particles together, creating stable aggregates that contribute to increased soil aeration, water infiltration, and water holding capacity of the soil, thereby enhancing soil resistance to erosion (Vogelsang et al., 2004; van der Wal et al., 2009). As a result, rhizospheric fungi are directly involved in soil fertility (Sterkenburg et al., 2015; Rashid et al., 2016) and contribute to the mitigation of soil degradation (Rashid et al., 2016; Rosas-Medina et al., 2020).

Among rhizospheric fungi, mycorrhizal fungi comprise one of the major groups since they are associated with more than 90% of known terrestrial plants (Smith and Read, 2008; Nilsson et al., 2019; Islam et al., 2022). Mycorrhizal fungi significantly improve the absorption and use of nutrients by host plants, stimulate growth, increase stress and disease resistance, and thereby contribute to maintaining the aboveground primary productivity of forest and ecosystem stability (Larekeng et al., 2019; Thind et al., 2022). According to root morphological differentiation, there are many types of mycorrhizal fungi of which one of them is ectomycorrhizal (EcM) fungi. They are obligate partners of most woody plant species that majorly belong to the families Fagaceae, Dipterocarpaceae, Phyllanthaceae, Myrtaceae, etc. (Brundrett and Tedersoo, 2018; Corrales et al., 2018). In tropical Africa, some EcM trees that belong to these families are *Afzelia africana* Smith ex Persoon, *Berlinia grandifolia* (Vahl) Hutch. and Dalziel, *Monotes kerstingii* Gilg, *Isoberlinia doka* Craib and Stapf, *Isoberlinia tomentosa* (Harms) Craib and Stapf, *Uapaca togoensis* Pax, etc. (Bâ et al., 2012; Houdanon et al., 2019). They are economically important trees and because of their socio-economic value, these species are facing major pressure from the local population, including charcoal production, and illegal logging for furniture (Balima et al., 2018; Mohammed et al., 2021). In addition, natural regeneration is not able to compensate for the removal of trees from the forest. Therefore, attempts to plant nursery-produced seedlings in the wild have been considered (Ogbimi et al., 2020; Ogbimi and Sakpere, 2021). However, since nursery production does not include knowledge of the niche of these plant species in their natural habitats, the results of planting in the wild are not satisfactory. Given that fungi play a key role in plant growth and health, there is a clear need to better understand the soil mycobiome surrounding native forest trees to develop an effective sustainable management strategy.

Until recently, studies on fungal diversity in West Africa have relied primarily on fruiting bodies surveys, mycelia isolations, and spore identification (Straatsma et al., 2001; Luo et al., 2020). Fruit bodies-based surveys do not allow a total evaluation of the fungal community (Kubartová et al., 2012; Shirouzu et al., 2016), because even if a fungus has basidiomata large enough to be spotted, they may go unnoticed because fruiting body formation is both seasonal and ephemeral (Shirouzu et al., 2016). Many taxa such as mycorrhizal and parasitic fungi may not grow or produce reproductive structures on artificial media even if they are potentially culturable (Allen et al., 2003; Senanayake et al., 2020). In addition to the aforementioned methods, spore identification is traditionally used to identify the rhizosphere arbuscular mycorrhizal fungi (Rodríguez-Morelos et al., 2014; Xavier and Rodrigues, 2020). However, even though this method is important in fungal taxonomy, it is time- and energy-consuming and susceptible to variability in spore morphology description, because host species and microbial age may be very challenging to differentiate spores of similar species (Bhat et al., 2014; Senanayake et al., 2020). Recent studies using high-throughput sequencing of environmental samples have greatly improved our understanding of the community and diversity of rhizosphere soil fungi (Tedersoo et al., 2014; Qin, 2018; Zhu et al., 2018; Nilsson et al., 2019; Tremblay et al., 2019; Meidl et al., 2021).

One of the most accepted methods for high throughput sequencing is the generation of the amplicon sequence variants (ASVs). So far, this method has been mainly used to study soil mycobiome in temperate and boreal regions (Wu et al., 2019; Lance et al., 2020; Rosas-Medina et al., 2020), while very few studies have comprehensively assessed the diversity, and community composition of soil fungi in tropical African forest ecosystems (Meidl et al., 2021). Here, PacBio sequencing was employed to assess the diversity and community composition of fungi found in the rhizosphere of five West African native trees.

MATERIALS AND METHODS

Study area

The soil samples used in this study were collected across five West African countries namely Benin, Burkina Faso, Guinea, Côte d'Ivoire, and Mali. In total, nine forests containing at least one of the targeted EcM tree species were selected. The different forests range from woodlands to gallery forests: The gallery forests and the woodland of Kota in Benin, the Kou gallery forest and the Niangoloko forest reserve in Burkina-Faso, the Farako1 forest reserve and the Farako15 forest reserve in Mali, the Bissandougou forest reserve and Moussaya forest reserves in Guinae and the Kouadianikro gallery forest in Côte d'Ivoire (Figure 1).

Sampling design and methods

Within each study site, we established a plot of 50 m × 50 m (2500

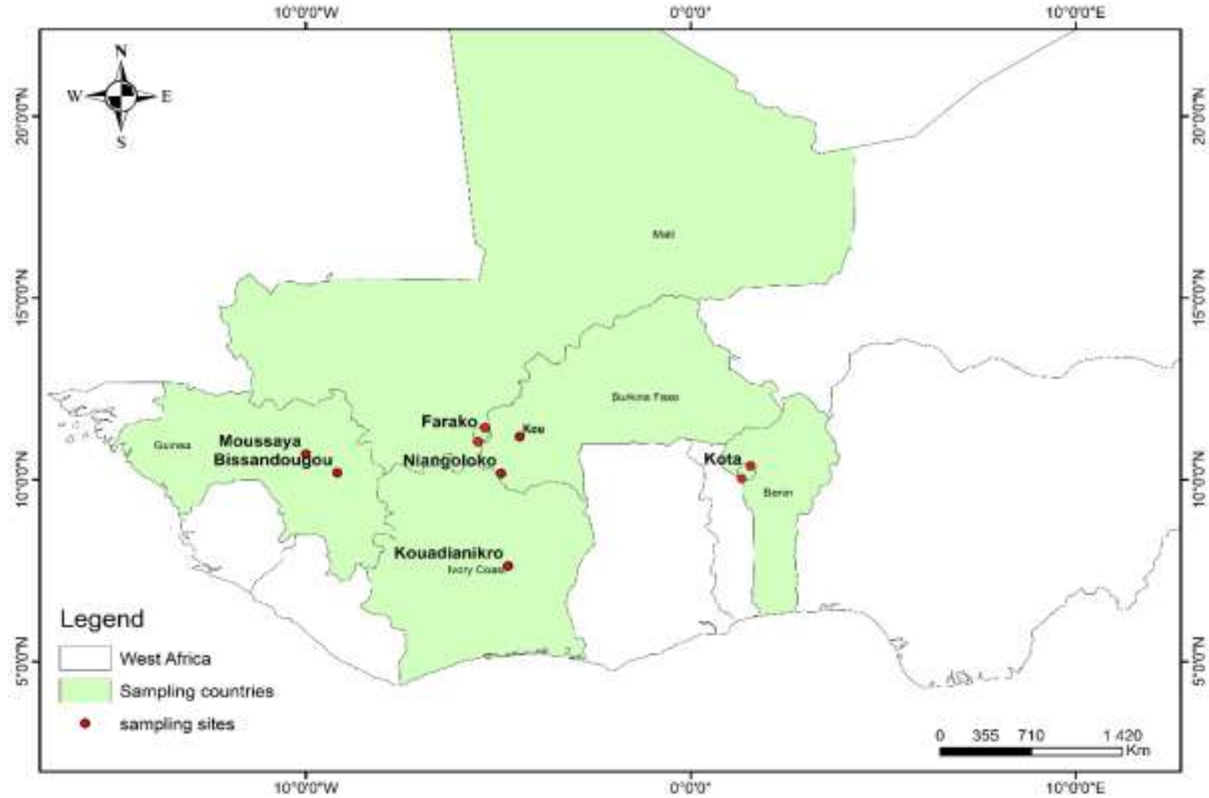

Figure 1. Study area and the sampling sites in red dots.
Source: Authors

m^2) in woodlands and a rectangular plot of 30 m × 80 m (2,400 m^2) within gallery forests due to their shape. Within each plot, five EcM trees were targeted, namely *I. doka*, *I. tomentosa*, *U. togoensis*, *M. kerstingii*, and *B. grandiflora*. Ten trees were chosen in proportion to their abundance, while ensuring that each of the EcM trees in the plot is represented at least once and that all sampled trees were at least eight meters apart. Under each targeted tree, two soil samples of about 200 g around 1 m was taken from each side of the trunk using a small shovel to collect the first 15 cm of soil. The two soil samples were pooled in a plastic bag. A total of 90 (5 EcM trees x 2 samples x 9 sites) soil samples were collected at a rate of 10 samples per site. Later on, the collected soil samples were processed following the protocol described by Tedersoo et al. (2014).

DNA extraction, sequencing and bioinformatics analyses

For the DNA extraction and sequencing, soil samples were sent to the Department of Ecology and Genetics, Evolutionary Biology, Uppsala University. A subsample of approximately 250 mg was placed in a separate 2.0 ml tube containing 750 µl of field lysis and preservation buffer (Xpedition Soil/Fecal DNA miniprep, Zymo Research Corporation, Irvine, California, USA) and lysed in the field using a portable bead beater (TeraLyser, Zymo Research Corporation).

Extraction, amplification, sequencing, and clustering of sequences into amplicon sequence variants (ASVs) were performed as described by Meidl et al. (2021). For more details, see the methodology of Meidl et al. (2021). The taxonomic attribution of the different ASVs was carried out on the PlutoF platform (Kõljalg et al., 2019) using the PROTAX software (Somervuo et al., 2016) (publication date 2020-10-21), configured by the Index Fungorum taxonomic database and the UNITE reference sequence database (Nilsson et al., 2016). We recorded for each query ASV the most likely taxonomic identity at the phylum, class, order, family, genus, and species levels, as well as the uncertainty of these assignments, measured by probabilistic placement. The authors note that the PROTAX uncertainty estimates explain the possibility that the species is unknown to science (that is, not included in the taxonomic database), or known to science but lacking sequences reference (Somervuo et al., 2016; Abarenkov et al., 2018).

Data processing and analysis

To illustrate the fungal taxonomic composition associated with the rhizosphere of the target EcM trees, we constructed a Krona wheel for each tree using Protax-fungi in PlutoF platform from ASV diversity. Alpha diversity was determined for each EcM tree by calculating species richness and the Shannon diversity index. The similarity analysis (ANOSIM) was used to assess the similarity between the fungal communities associated with EcM trees. Through principal component analysis, we highlight fungal species affiliation with each EcM tree, and to identify the potential fungal species which better characterize each EcM tree. Finally, the Jaccard similarity index was calculated to compare the proportion of species shared by different EcM trees. All these analyses were carried out using the vegan package (Oksanen et al. 2022) with the statistical software R version 3.6.2 (R Core Team, 2019) and the ggplot2 package (Wickham, 2016) was used to create the nMDS graph.

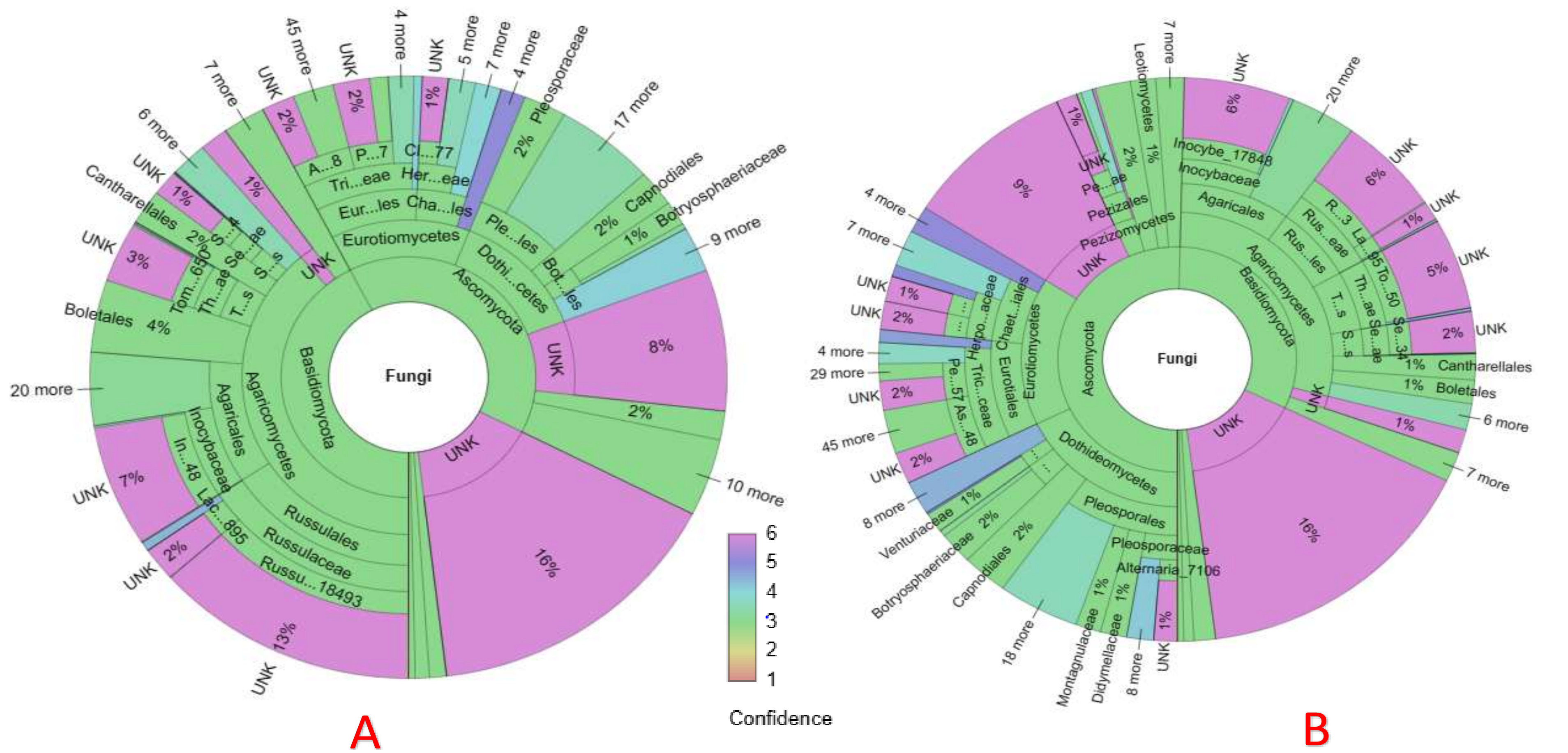

Figure 2. Krona-Wheels illustrating the taxonomic distribution of fungi in soil samples. Results of samples associated with the rhizosphere of *Berlinia grandiflora* (A) and *Isoberlina doka* (B).
Source: Authors

RESULTS

Taxonomic composition of fungal communities associated with the rhizosphere of targeted EcM trees

Grouping the sequences into amplicon sequence variants (ASVs) gave a total of 1147 ASVs. In sum, 1051 ASV (91.63%) were identified as fungi. On the Krona wheels (Figures 2 to 4, Supplementary materials A, B, C, D and E for more detail), the color scales show the type and confidence level of each taxonomic placement. Color scales 1 to 3 correspond to the identified taxonomic units for which the proportion of reliable identifications ranges from 50… 100% (1), 0… 50% (2) or 0 % Color 3. Scales 4 to 6 correspond to unknown taxonomic units. In total, four taxonomic groups of fungi such as Basidiomycota, Ascomycota, Glomeromycota, Zygomycota were identified from the rhizosphere of the targeted

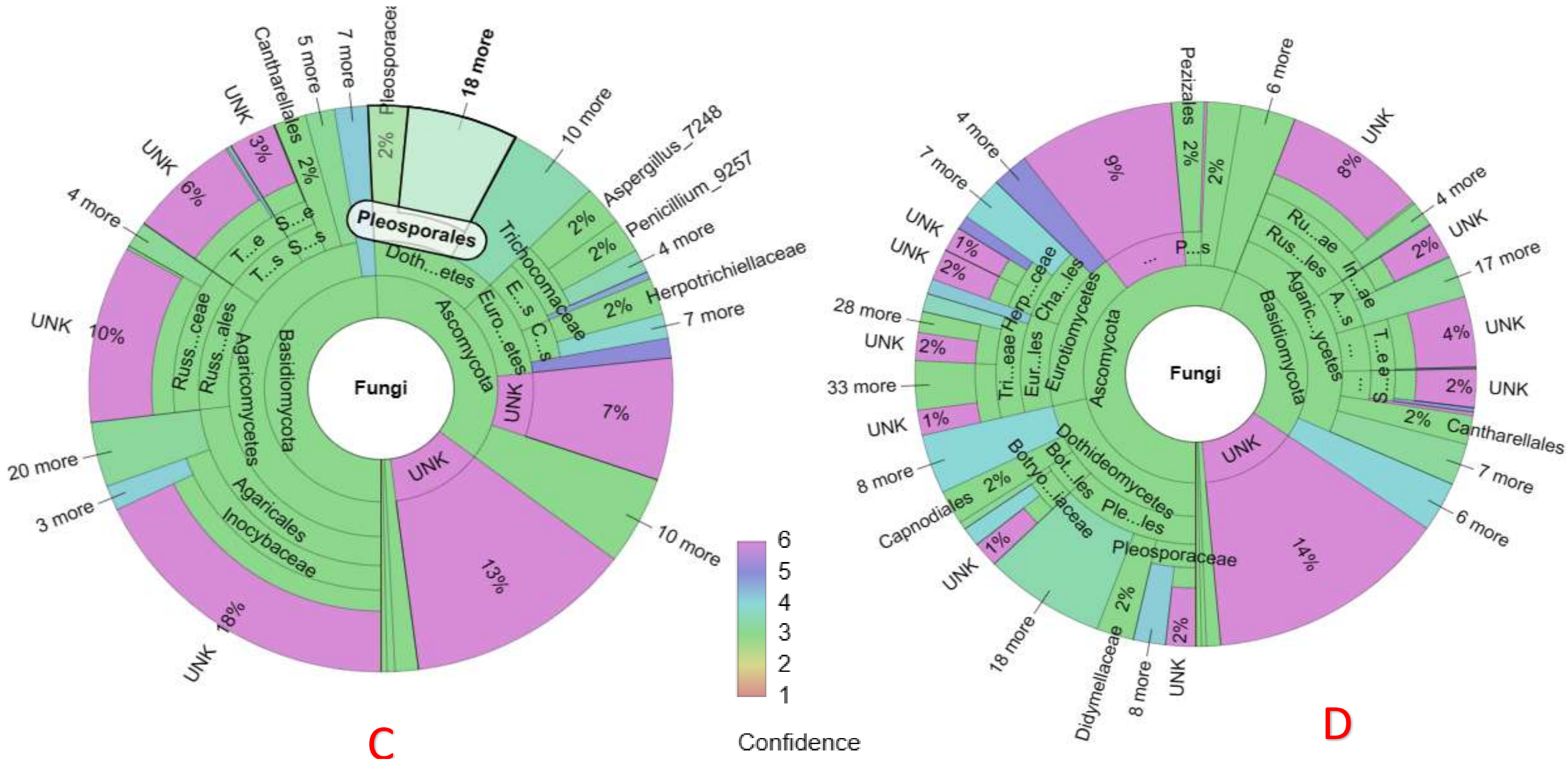

Figure 3. Krona-Wheels illustrating the taxonomic distribution of fungi in soil samples. Results of samples associated with the rhizosphere of *Isoberlina tomentosa* (C) and *Monotes kerstingii* (D).
Source: Authors

EcM trees. These latter are unevenly distributed for each EcM tree. For example, Basidiomycota are most dominant under *B. grandiflora* (42%) and *I. tomentosa* (49%); while Ascomycota are the most dominant under *I. doka* (50%), *M. kerstingii* (56%), and *U. togoensis* (50%). Glomeromycota and Zygomycota are weakly represented under the target EcM trees. Sixteen percent of the sequences associated with *B. grandiflora* and *I. doka* are unidentified or unknown. Fourteen percent of the fungi sampled under *M. kerstingii* and *U. togoensis* are unidentified, whilst unknown taxa make up to 13% of total fungal community under *I. tomentosa.*

In general, *Russulales* is the most dominant fungal group under *B. grandiflora*, *I. doka*, *U. togoensis*, and *M. kerstingii,* while *Agaricales* is more abundant in the rhizosphere of *I. tomentosa* (Figure 5). Sebacinales are more represented under *M. kerstingii* than the other trees investigated in contrast to Boletales, which are

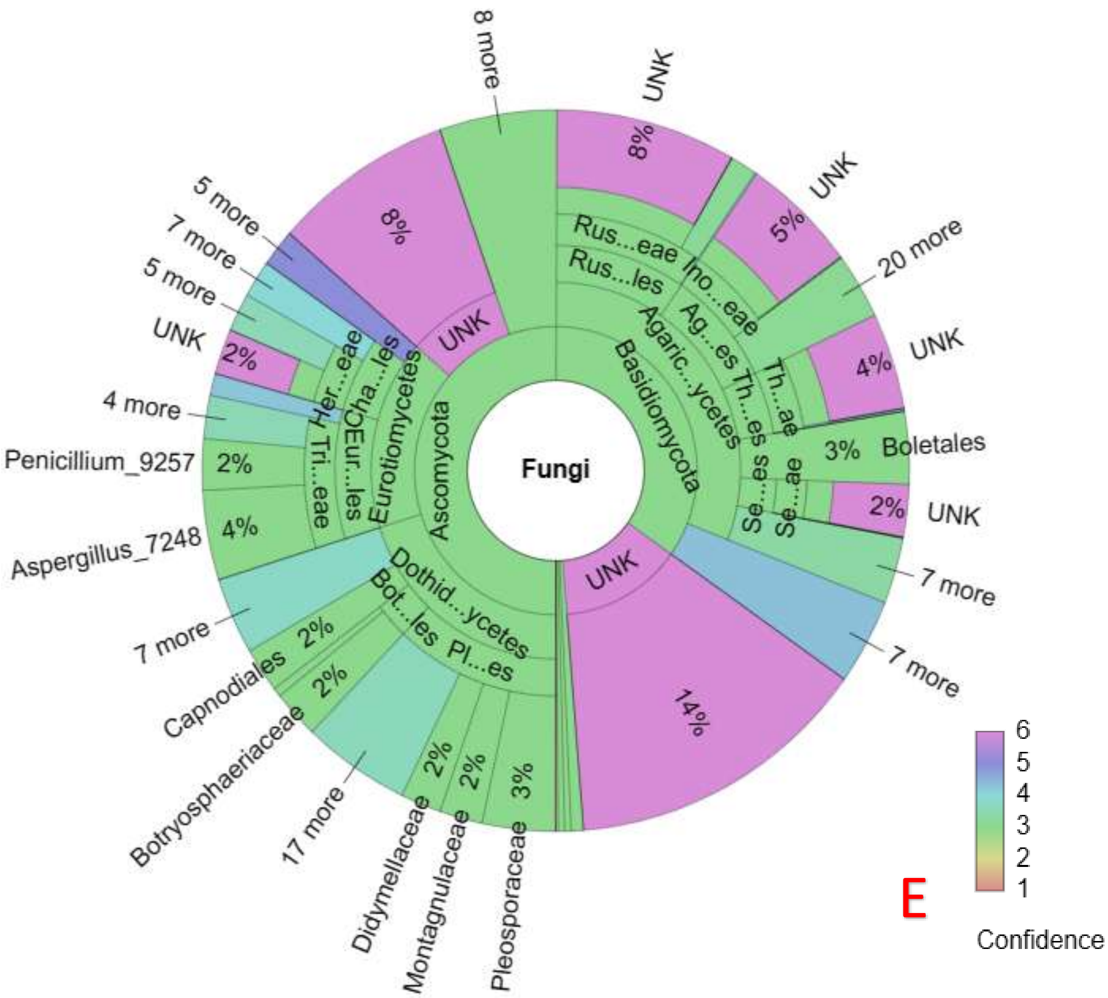

Figure 4. Krona-Wheels illustrating the taxonomic distribution of fungi in soil samples. Results of samples associated with the rhizosphere of *Uapaca togoensis* (E).
Source: Authors

more represented under *B. grandifolia*. *I. doka* and *I. tomentosa* have the highest proportion of Pezizales. Cantharellales, an important group of edible fungi in tropical Africa, is best represented under *I. tomentosa*.

Genera representativeness under the different forest species

A total of 1051 ASV, including 810 (77.07%) belonging to 90 species from 84 genera, 71 families, 40 orders, 19 classes, and 04 phyla have been recorded. Moreover, 66.67% of this specific richness is observed under *B. grandiflora* (60 species), against 62.22% for *I. doka* (56 species), 53.33% for *I. tomentosa* (48 species), 48.89% for *U. togoensis* (44 species), and 47.78% for *M. kerstingii* (43 species). The real diversity is probably much higher because about 60% of the genera (50 genera for all EcM trees combined) could not be identified up to species level. About 22.93% (241) of the ASV remained unidentified and were not included in this analysis. *Russula* is better represented under *B. grandiflora*, *I. doka*, *U. togoensis*, and *M. kerstingii* unlike *Inocybe* that is much more observed under *I. tomentosa* (Figure 6).

Diversity of belowground fungal communities of five EcM trees

Table 1 presents the intraspecific diversity of the belowground fungal communities of the different tree species in the EcM. At the genus level, the belowground fungal communities were found to be the most diverse for *Isoberlinia doka* (G = 63, H' = 2.81, J = 0.679) and the least diverse for *Monotes kerstingii* (G = 54, H' = 1.78, J = 0.447). On the other hand, fungal generic diversity affiliated with *Uapaca togoensis* (G = 53, H' = 2.39, J =

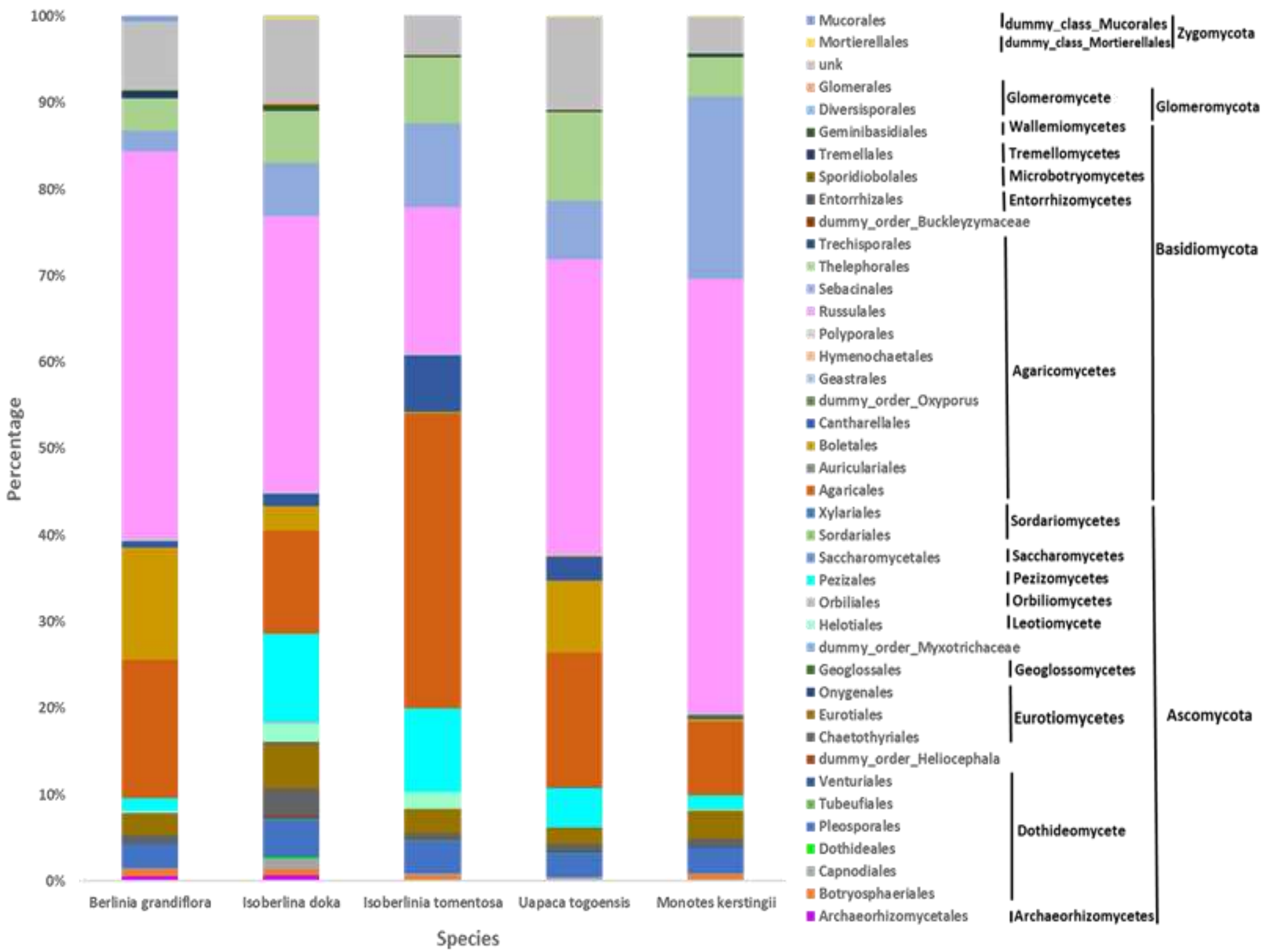

Figure 5. Representativeness of fungal taxa under target forest species.
Source: Authors

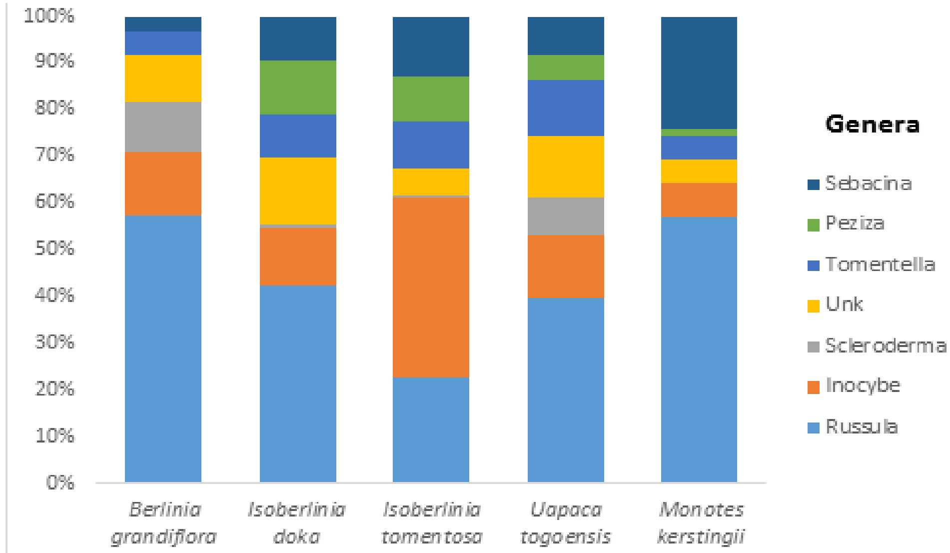

Figure 6. Distribution of the best-represented genera within the different EcM tree species.
Source: Authors

Table 1. Genus level intraspecific diversity of belowground fungal community of ectomycorrhizal host trees.

Forest trees	Richness	Shannon	Evenness
Isoberlinia doka	63	2.81	0.679
Isoberlinia tomentosa	62	2.48	0.6
Uapacca togoensis	53	2.39	0.603
Berlinia grandiflora	67	2.24	0.533
Monotes kerstingii	54	1.78	0.447

Source: Authors

Table 2. Similarity index of Jaccard among the forest trees.

Species	*I. doka*	*I. tomentosa*	*M. kerstingii*	*B. grandiflora*
I. tomentosa	0.831			
M. kerstingii	0.692	0. 692		
B. grandiflora	0.658	0.725	0.725	
U. togoensis	0.635	0.676	0.700	0.800

Source: Authors

0.603) was approximately equal to that of *Isoberlinia tomentosa* (G = 62, H' = 2.48, J = 0.6).

Considering pairwise EcM trees, Jaccard's similarity index (Table 2) indicated generally large proportions of shared fungal genera. Indeed, similarity (0.635) was obtained between *I. doka* and *U. togoensis;* but *I. doka* and *I. tomentosa* shared the largest number of taxa (Jaccard index = 0.831). Although the proportion of genera shared was greater than 0.6 in all pairwise cases, the similarity analysis (ANOSIM) supported the evidence that at the genus level, the belowground fungal community associated with the rhizosphere of at least one of the five EcM trees differed significantly from the others ($P < 0.05$, Figure 7).

Categorization of below-ground fungal species according to EcM hosts

Figure 8 presents the projection of fungal genera

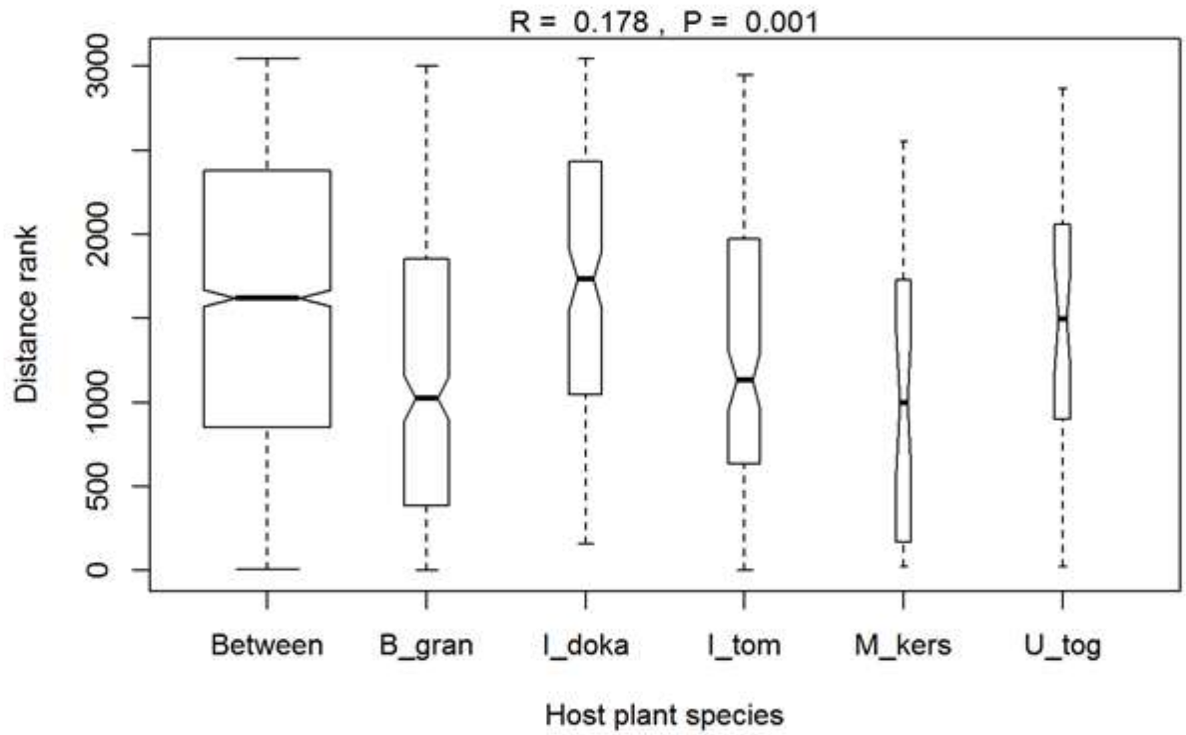

Figure 7. Similarity distance between the compositions of the fungal microbiome found under forest species. (B_gran) *Berlinia grandiflora*; (I_doka) *Isoberlinia doka*; (I_tom) *Isoberlinia tomentosa*; (M_kers) *Monotes kestingii*; (U_tog) *Uapaca togoensis.*
Source: Authors

generated for each EcM tree according to the principal components 1 and 2. Figure 8 suggests that EcM trees hardly cluster separately and share a large number of fungal genera as the similarity index of Jaccard indicated it. This makes it difficult to clearly identify the genera that characterized the fungal community of each tree. Nevertheless, through the projection of the circles, the genus *Russula* seems to cluster more with *B. grandiflora;* while *Sebacina* seems more associated with *M. kerstingii* and *I. tomentosa*; and the genus *Inocybe* clusters more with *I. tomentosa*.

DISCUSSION

To assess the diversity and community composition of fungi found in the rhizosphere of five West African native trees, high throughput sequencing was employed. Out of 1051 ASVs generated, a significant percentage of 22.93% remained unidentified. This could potentially be explained by the incompleteness of the reference databases or taxonomic placement (Somervuo et al., 2016; Abarenkov et al., 2018). Secondly, the high percentage of unknown taxa suggests that a large proportion of taxa remain to be described. In a global study on soil fungi, Tedersoo et al. (2014) estimate that about 80% of all soil fungal taxa cannot be identified to the species level, and 20% reliably assigned to known orders. The data, therefore, opens new perspectives for future work on the analysis of undescribed or at least not yet sequenced fungal species, the estimation of below-ground fungal diversity and therefore calls for a greater sampling effort in West African soils (Crous et al., 2006). Basidiomycota are better represented under *I. tomentosa* (49%) and *B. gandiflora* (42%); unlike the Ascomycota that are more recorded under *M. kerstingii* (56%), *I. doka* (50%), and *U. togoensis* (50%). Also, the genus *Russula* is most abundant under *B. grandiflora*, *I. doka*, *U. togoensis*, and *M. kerstingii;* unlike *Inocybe* that is more frequently observed under *I. tomentosa*. These results largely corroborate previous observations that EcM fungal communities in West Africa are dominated by fungi in Russulaceae families (Bâ et al., 2012; Tedersoo and Smith, 2013, 2017; Ebenye et al., 2017). Meild et al. (2021) also reported the dominance of the genera *Russula* and *Inocybe* in the same geographical areas. The high proportion of Ascomycota (*Peziza*) in the soil fungal community highlights the presence of trophic groups other than EcM and their potential role as important decomposers of a wide variety of dead organic matter in forest ecosystems through the production of a wide range of hydrolytic enzymes, including cellulase and

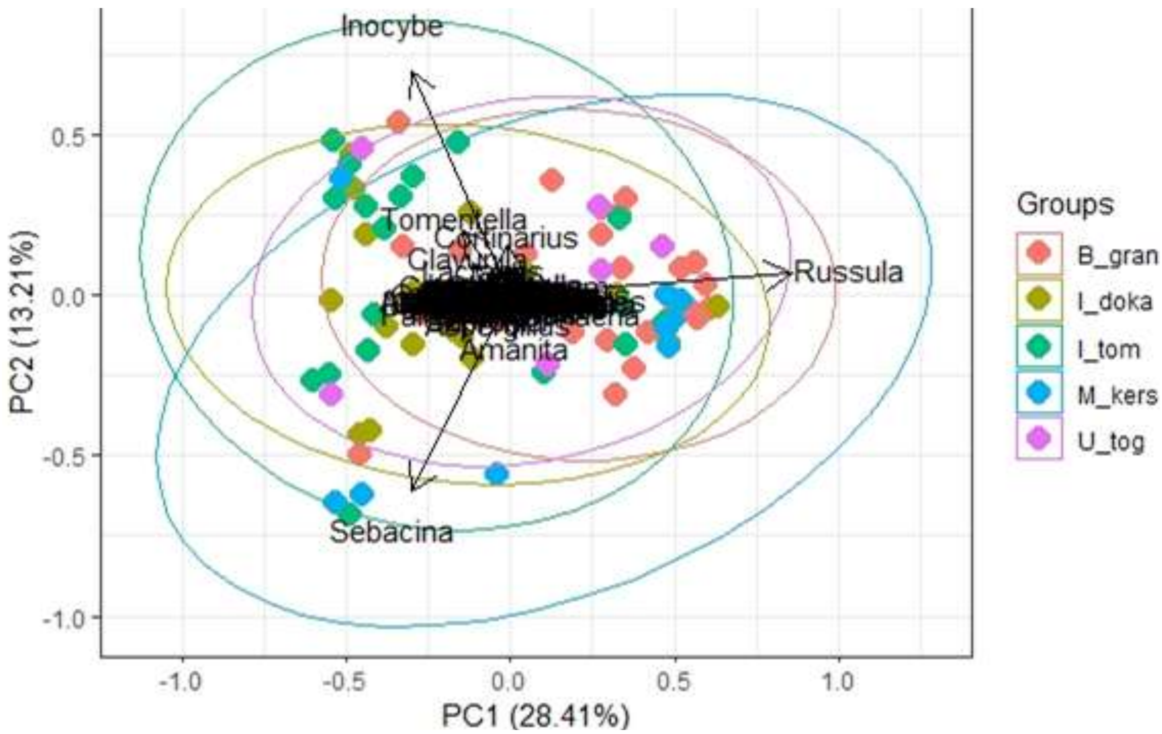

Figure 8. Prioritization of fungal species according to the EcM tree species.
Source: Authors

phenol oxidases (Egger, 1986). The absence or low representativeness of certain groups of fungi with large fruit bodies such as Polyporales and Hymenochaetales, has also been evidenced regarding soil fungi in temperate ecosystems (Tedersoo et al., 2020). This suggests a general pattern indicative of soil fungal communities and a limitation of exchange between the fungal communities of the phyllosphere and dead wood within the soil. Moreover, the effective presence of Glomeromycota highlights the probable duality of EcM and AMF of these trees. It has been demonstrated that some local forest trees form both EcM and AMF symbioses (Houngnandan et al., 2009; Djotan et al., 2021).

The diversity indices indicate a higher species diversity for *Isoberlinia doka* (G = 63, H' = 2.81, J = 0.679). For the other forest species (*I. tomentosa*; *U. togoensis*; *M. kerstingii* and *B. grandiflora*), the diversity is low with an average distribution between genera. Fonton et al. (2012) argued that *I. doka* is a good early colonizer because it can reproduce from suckers and grows quickly. As such, *I. Doka* can connect to a larger number of below-ground fungal networks (Diédhiou et al., 2010; Gorzelak et al., 2015; Mcguire, 2017). Also, the density or uneven distribution of stands dominated by target EcM trees could explain this observation, but also other factors including different soil characteristics, altitude, and host specificity (Corrales et al., 2018). Indeed, the increasing proportion of phosphorus, clay, nitrogen, and soil pH, is negatively correlated with fungal community diversity, abundance, and composition (LeDuc et al., 2013; Zhang et al., 2016). This difference in belowground fungal community diversity among EcM trees is strongly correlated with canopy composition, stand age, EcM tree density, and canopy cover rate (Johnson et al., 2004; Gebhardt et al., 2007; Burke et al., 2009; Henry et al., 2021; Meidl et al., 2021). However, the Jaccard similarity index shows that a large proportion of genera are shared. *I. doka* and *I. tomentosa* share the greatest number of common genera (J = 0.831); unlike *I. doka* and *U. togoensis,* which display the lowest number (J = 0.635). *I. doka* and *I. tomentosa* are two EcM sister species within the same family (Fabaceae). Such phylogenetic proximity could explain why both tree species obtained the highest value of the similarity index. However, the similarity analysis (AnoSim) indicates that the generic fungal composition differs significantly between the five EcM trees (P = 0.001) at the 5% level.

Based on nMDS results, only three of the fungal genera are more specific to certain woody species. This could be explained by the preference or specificity of certain fungal partners in symbiotic relationships with EcM trees. Previous studies highlighted the close preference between certain bellowground fungal communities and their host plants (Kretzer et al., 1996; Taylor and Bruns, 1997; Taylor et al., 2002). For example, *Lactarius deliciosus* (L.) Gray, *L. deterrimus* Gröger and *L. salmonicolor* R. Heim and Leclair are specific to *Pinus* *sylvestris* Baumg., *Picea abies* (L.) H.Karst. and *Abies alba* (Aiton) Michx., respectively (Giollant et al., 1993). Still, the specificity of this fungal community is closely linked to a genus or family of partner plants (Massicotte et al., 1994; Molina and Trappe, 1994). These results corroborate those of Toju et al. (2013), who pointed out that some fungi of the Russulaceae family have been detected exclusively on specific oak species (*Quercus* spp.). Other research confirms the specificity of some genera of soil fungi with respect to their host plants (Ishida et al., 2007; Tedersoo et al., 2008). This is the case for fungal species such as *Rhizopogon* spp. and *Suillus* spp., which are almost exclusively associated with Pinaceae and sometimes Monotropaceae (Massicotte et al., 1994; Molina and Trappe, 1994; Kretzer et al., 1996; Taylor and Bruns, 1997; Taylor et al., 2002).

While the recent work of Meidl et al. (2021) aimed to document the effect of vegetation types on the mycobiome of soils associated with EcM trees, the present study targets the relation between selected EcM trees and the mycobiome immediately within their rhizosphere (all vegetation combined). The findings corroborate previous work by Massicotte et al. (1994), Molina and Trappe (1994), Kretzer et al. (1996), Taylor and Bruns (1997), Taylor et al. (2002), Ishida et al. (2007), Tedersoo et al. (2008), which highlighted different mechanisms of microbiome specification by host plants. The results, therefore, supplement those of Meild et al. (2021) not only by confirming host preference but more importantly by highlighting the specialist genera partnered with valuable native tree species of West Africa.

Conclusion

Until recently, estimates of total fungal diversity did not include results from large-scale environmental sequencing methods, especially in West African regions. This study constitutes the first major exploration of the edaphic fungal communities of West African ecosystems, revealing insufficient sampling effort in currently neglected ecosystems and regions. The authors' data provide a baseline for phylogenetic placement and taxonomic resolution of environmental sequences of five EcM trees of socio-economic importance in West Africa.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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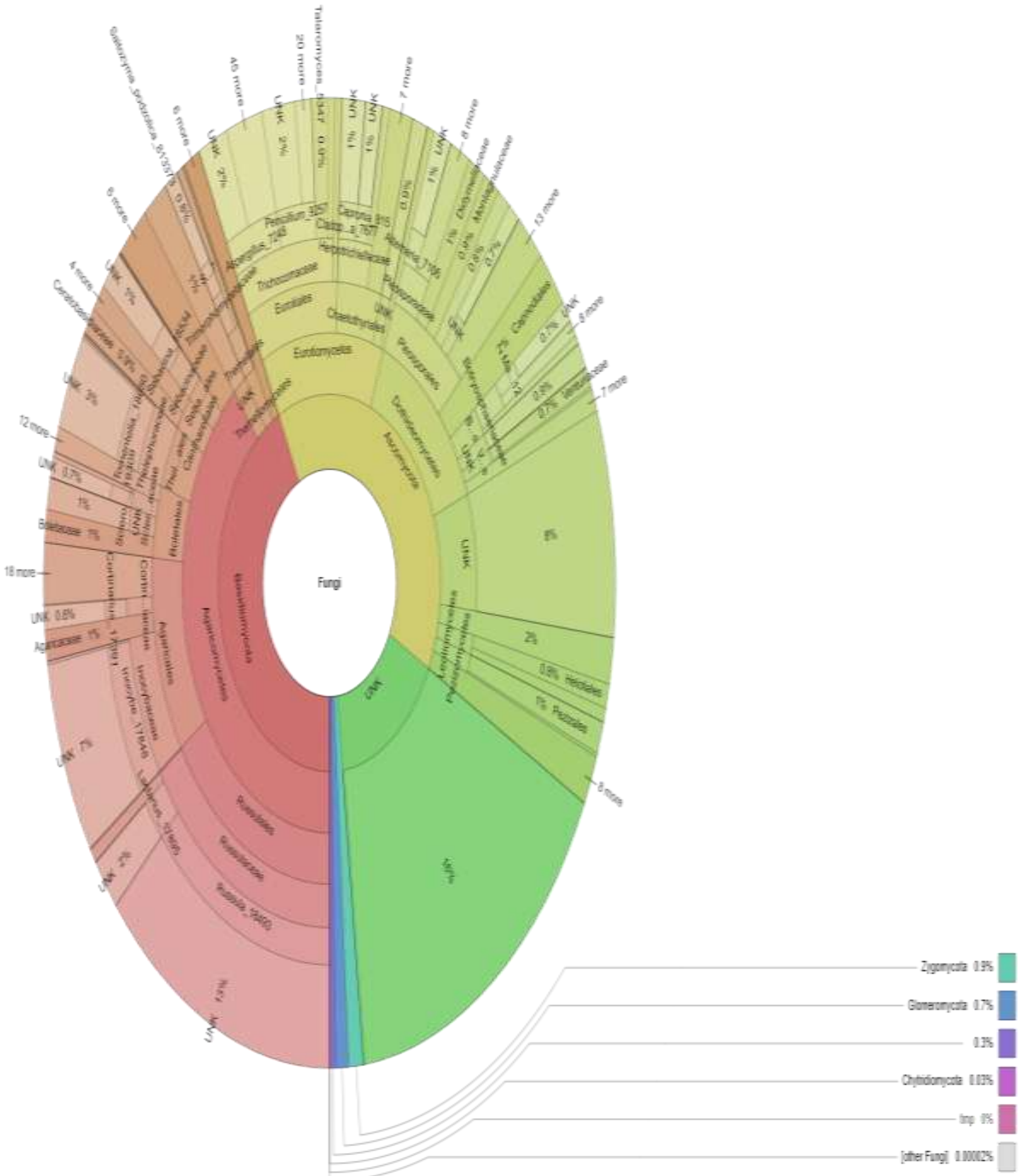
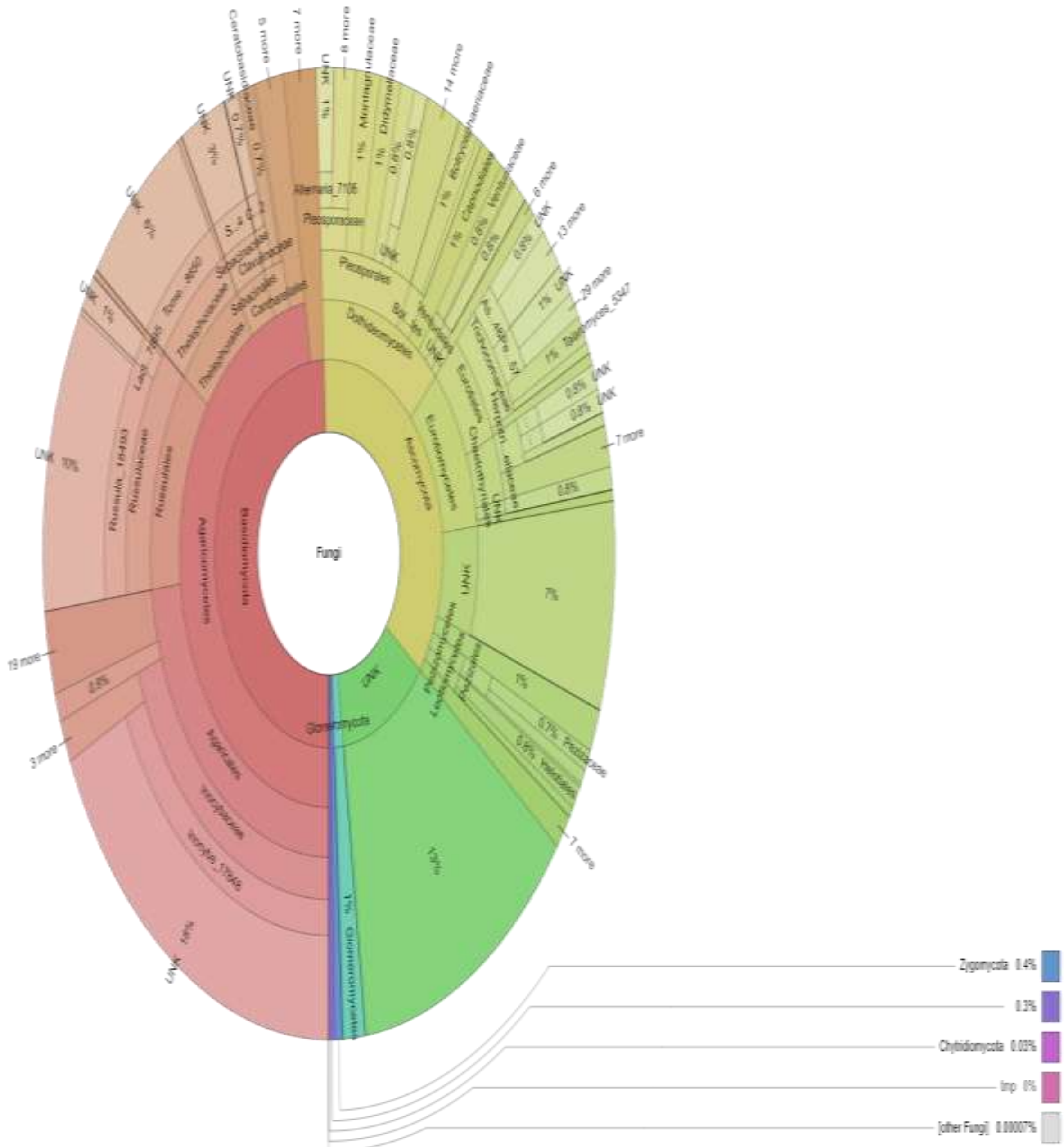


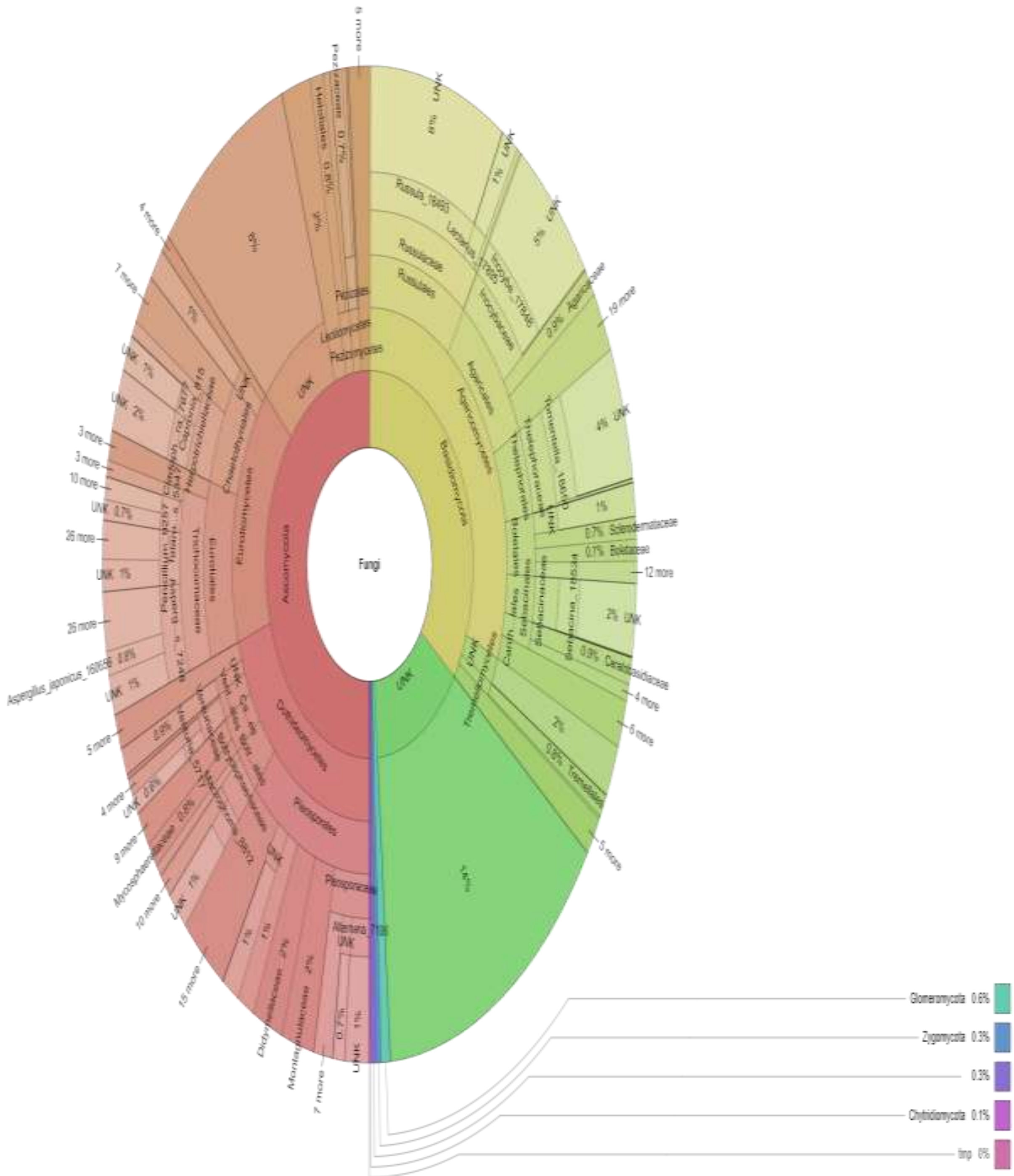
Supplementary materials A

Supplementary materials B



Supplementary materials C

Supplementary materials D



Supplementary materials E

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