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Title: Impact of intercropping on the coupling between soil microbial community structure, activity, and nutrient-use efficiencies

Tengxiang Lian[1,2], Yinghui Mu[1,2], Jian Jin[3], Qibin Ma[1,2], Yanbo Cheng[1,2], Zhandong Cai[1,2], Hai Nian[1,2]*

1.The State Key Laboratory for Conservation and Utilization of Subtropical Agro-bioresources, South China Agricultural University, Guangzhou 510642, Guangdong, People's Republic of China

2.The Key Laboratory of Plant Molecular Breeding of Guangdong Province, College of Agriculture, South China Agricultural University, Guangzhou 510642, Guangdong, People's Republic of China

3.Key Laboratory of Mollisols Agroecology, Northeast Institute of Geography and Agroecology, Chinese Academy of Sciences, Harbin 150081, China

* **Corresponding author:** Hai Nian

Corresponding address: No.483 Wushan Road, Guangzhou, Guangdong, 510642, China.

Tel.: +8602085288024

Fax: +8602085288024

E-mail address: hnian@scau.edu.cn

Abstract

Sugarcane-soybean intercropping has been widely used to control disease and improve nutrition in the field. However, the response of the soil microbial community diversity and structure to intercropping is not well understood. Since microbial diversity corresponds to soil quality and plant health, a pot experiment was conducted with sugarcane intercropped with soybean. Rhizosphere soil was collected 40 days after sowing, and MiSeq sequencing was utilized to analyse the soil microbial community diversity and composition. Soil columns were used to assess the influence of intercropping on soil microbial activity (soil respiration and carbon-use efficiency : nitrogen-use efficiency ratio). PICRUSt and FUNGuild analysis were conducted to predict microbial functional profiling. Our results showed that intercropping decreased pH by approximately 8.9% and enhanced the soil organic carbon (SOC), dissolved organic carbon (DOC), and available nitrogen (N) by 5.5%, 13.4% and 10.0%, respectively. These changed changes in physiochemicalphysicochemical properties triggered thecorresponded to increased microbial diversity and shifted shifts in soil microbial communities. Microbial community was correlated significantly ($p < 0.05$) correlated towith microbial activity that reflected in higher soil respiration rates and nutrient use efficiency in the intercropping system. Furthermore, intercropping influenced microbial functions, such as carbon fixation pathways in prokaryotes, citrate cycle (TCA cycle) of bacteria and wood saprotrophs of fungi. These overrepresented functions might accelerate nutrient conversion and control phytopathogens in soil.

Keywords: Sugarcane-soybean intercropping; microbial community structure; carbon-use efficiency; nitrogen-use efficiency

1. Introduction

Sugarcane-soybean intercropping has been widely used to stabilize yields and reduce nitrogen leaching (Edwin et al., 2005; Xu et al., 2008; Li et al., 2013). ~~Soybean with~~ N fixation ~~capacity,~~associated with soybeans~~ which~~ can improve soil fertility and field ecological conditions~~, are favourable for~~ that favor sugarcane in the intercropping system (He et al., 2006). Intercropping of sugarcane with soybean, may also stimulate N fixation by the legume's microbiome (Li et al., 2013).

In an intercropping system, the roots of different plant species interact directly with each other and subsequently affect root exudation, which undoubtedly alters the microbial diversity, structure, and activity (Zhou et al., 2011; Broeckling et al., 2008; Gomes et al., 2003). The changed microbial community and activity by intercropping could affect C and N dynamics (Kaur et al., 2000; Rowe et al., 2005; Sun et al., 2009), and this may be attributed to the ability of microbial communities to regulate carbon and nitrogen-use efficiency to maintain resource balances (Mooshammer et al., 2014). Thus, a comprehensive method that incorporates the carbon-use efficiency : nitrogen-use efficiency ratio and soil respiration could be used to evaluate the change in microbial activity caused by the microbial community (Zhong et al., 2015).

The influence of intercropping on the soil microbial communities in several intercropping systems have been studied, such as mulberry–soybean, *Eucalyptus–Acacia mangium* and apple tree-crown vetch intercropping (Li et al., 2013; Li et al., 2016; Rachid et al., 2015, Zheng et al., 2018). For example, Li et al. (2016) investigated the effects of mulberry–soybean intercropping

on the diversity and composition of the soil bacterial community in salt–alkali soil and found that the bacterial diversity and structure varied between monoculture and intercropping treatments. Among the bacteria, some phosphate-solubilizing bacteria, such as *Burkholderia*, *Arthrobacter,* and *Pseudomonas*, were more abundant in both soybean- and mulberry-grown soil in the intercropping system. Moreover, Rachid et al. (2015) reported that *Eucalyptus* intercropped with *Acacia mangium* increased soil fungal community diversity and changed the fungal structure, and they observed some frequency of several genera that were not found in the monoculture cultivation samples. For apple tree intercropped with crown vetch, soil bacterial community structure differed with intercrop and monoculture treatment, although bacterial richness and diversity was not impacted (Zheng et al., 2018).

In our study, the bacterial and fungal structure and activity in the intercropping and monoculture system were analysed. We hypothesized that intercropping improves soil properties, increases the microbial diversity, changes community structure and improves some microbial function (H1) and that change in microbial community will correlate with microbial activity (H2). Our results could provide insight into how intercropping management improve soil properties and microbial activity compared to monoculture.

2. Materials and methods

2.1 Experimental design and plant materials

The intercropping experiment was established in March of 2016 with three replicates of three treatments in a randomized block design. The treatments included (1) sugarcane monoculture, (2) soybean monoculture and (3) sugarcane intercropped with soybean. The soil used in this

study was classified as Ali-Udic Argosol with pH 5.1, soil organic carbon (SOC) 8.5 g kg^{-1}, 0.41 g kg^{-1} total N and 0.42 g kg^{-1} total P.

The sugarcane variety ROC22 (*Saccharum officinarum*) and soybean variety HuaChun5 (*Glycine max L.*), which are widely grown in South China, were used in this study. Plants were grown in pots in the glasshouse at South China Agriculture University, Guangzhou, China. In brief, all plants within a pot (140 cm wide × 45 cm width × 45 mm high) were filled with 30 kg of sieved soil (< 2 mm) and considered as one replicate. Two sugarcane seedlings or three soybean seeds were planted in a pot under the monoculture system, or two sugarcane seedlings with three soybean seeds were planted under the intercropping system. The row space was 0.9 m for sugarcane and 0.3 m for soybean in all treatments. The water content of the soil was adjusted to 80% of field water capacity. Plants were harvested at the flowering stage.

2.2. Soil sampling and measurements

Rhizosphere soil was recovered separately on 25 May 2016 (40 days after sowing) by shaking root for 3 min into a bag and mix thoroughly~~,~~. ~~and c~~Contact between samples was avoided. Approximately 5 g soil from each treatment was collected and stored at -80 °C for DNA extraction. Additionally, 100 g soil was collected and stored at 4 °C for analyses of microbial and soil ~~physiochemical~~physicochemical properties.

Soil pH was determined in a soil-water slurry (1:5 w:v) using a pH meter (FE20-FiveEasy™ pH, Mettler Toledo, German). Soil total nitrogen was measured using an elemental analyser (VarioEL III, Germany). Nitrate (NO_3^-) and ammonium (NH_4^+) were assayed using a continuous flow analytical system (SKALAR SAN++, The Netherlands). Soil organic carbon

104 (SOC), dissolved organic carbon (DOC), and dissolved organic nitrogen (DON) were measured
105 using a TOC analyser (Multi N/C 2100, Analytik Jena, Germany). Soil microbial biomass
106 carbon (MBC) and microbial biomass nitrogen (MBN) were measured by the chloroform-
107 fumigation extraction method (Vance et al., 1987). The sum value of NO_3^-, NH_4^+ and DON was
108 considered as available nitrogen (available N).

109 2.3. Soil incubation and respiration measurements

110 In brief, 20 g soil was collected from each treatment and placed into PVC cores (5 cm height,
111 2.5 cm diameter). The methods of the incubation experiment were reported in our previous
112 study (Lian et al., 2016). Briefly, the PVC core and a beaker with 10 ml 1M NaOH, which used
113 to trap CO_2, were placed into a 0.5-L sealed container. The tapped CO_2 was precipitated with
114 0.5 M $SrCl_2$ and and NaOH was neutralized with 0.1 M HCl ~~were used to precipitate the trapped~~
115 ~~CO_2 and neutralize NaOH, respectively~~. Soil respiration was estimated on 1, 3, 5, 7, 9, 11, 14,
116 18, 22, 26, 32, 39, 46, 53, and 60 days after incubation was initiated (Blagodatskaya et al. 2011).

117 2.4. DNA extraction and quantitative PCR (qPCR)

118 DNA was extracted using Fast DNA SPIN Kit for Soil (Qbiogene Inc., Carlsbad, CA, USA)
119 according to the manufacturer's instructions. Quantitative PCR (qPCR) was conducted by
120 targeting bacterial 16S rRNA genes and fungal ITS1 region, using the primers 515F/907R
121 (Osburn et al., 2011) and ITS1F/ITS2R (Yao et al., 2017), respectively, following the protocols
122 reported previously (Liu et al. 2015).

123 2.5. Illumina MiSeq sequencing analysis

Illumina MiSeq sequencing of the 16S rRNA genes and fungal ITS1 region was performed to examine the structure of the soil bacterial and fungal community, respectively. The raw sequences were processed and analysed using QIIME1 Pipeline Version 1.9.0. Multiple steps were conducted to remove low-quality sequences with lengths shorter than 200 bp and quality scores less than 20. For further analysis, the chimeric sequences were checked and removed using UCHIME algorithm. High-quality sequences were clustered into operational taxonomic units (OTUs) using RDP Classifier based on 97% sequence similarity. The OTUs were analysed using the SILVA and UNITE database for bacteria and fungi, respectively. Then, a phylogenetic tree was built using Fast Tree (Price et al., 2009). For a correct comparison between samples, ~~the lowest~~rarefied subsequening numbers (14811 for bacterial and 29726 for fungi) were used for subsequent analysis. All sequences have been deposited into the GenBank short-read archive under accession SRP116883 (bacteria) and SRP129902 (fungi).

2.6. Statistical analysis

Using the program R (version 3.4.4, vegan package), principal coordinate analysis (PCoA) based on OTU level was processed to assess the patterns of similarity (Bray-Curtis similarity) in the composition of the microbial community between treatments. The Chao 1 index and Shannon richness were calculated to compare soil bacterial and fungal alpha diversity (Fig. S1). A canonical correspondence analysis (CCA) was conducted to reveal the association between soil property variables and microbial community structure. Spearman correlation analysis was conducted with SPSS 24.0 to identify correlation between microbial activity and structure. PICRUSt analysis and STAMP were conducted to predict and visualize bacterial functional

profiling (Langille et al., 2013; Parks and Beiko, 2010). FUNGuild was used to identify fungi functional guilds (Nguyen et al., 2016).

ANOVA test was used with Genstat 13 (VSN International, Hemel Hemspstead, UK) to assess the effect of treatments on the SOC, total N, MBC, MBN, DOC, DON, NH_4^+-N, NO_3^--N, pH, and the relative abundance of OTU inferred with FUNGuild. Furthermore, ANOVA test of least significant difference (LSD) was used to assess the different ~~on~~ of respiration rate and cumulative respiration. Differences were considered statistically significant at level of $p < 0.05$. The ratios of microbial community carbon-use efficiency and nitrogen-use efficiency were calculated as follows (Mooshammer et al., 2014):

Carbon-use efficiency : Nitrogen-use efficiency = $B_{C:N}$: $R_{C:N}$

where $B_{C:N}$ is the C:N ratio of the microbial community and $R_{C:N}$ is the C:N ratio of the soil.

3. Results

3.1 Effect of intercropping on soil ~~physiochemical~~physicochemical properties

Compared with monoculture treatments, intercropping ~~resulted in a~~ decreased pH ~~decrease~~ from 6.73 to 6.13 and from 5.97 to 5.45 for sugarcane and soybean, respectively. The SOC was higher in I-Sugarcane (intercropped sugarcane) and I-Soybean (intercropped soybean) than that in the M-Sugarcane (sugarcane monoculture) and M-Soybean (soybean monoculture). The concentration of SOC significantly increased under I-Sugarcane compared with that under M-Sugarcane. NH_4^+, DOC, DON, MBC, and MBN levels were significantly increased for the two plant species under intercropping compared with those for the monoculture ($p < 0.05$), while

the NO_3^- level showed an opposite trend ($p < 0.05$) (Table 1).

3.2. Effect of intercropping on microbial activity

Soil respiration was enhanced in the intercropping system (Fig. 1). During the incubation, the cumulative CO_2-C levels were 6.9% and 5.3% greater in the intercropping soil than those in the monoculture for sugarcane and soybean, respectively (Fig. 1b). Moreover, the I-Sugarcane and I-Soybean treatments showed higher ratios of carbon- and nitrogen-use efficiency than that for the M-Sugarcane and M-Soybean, respectively (Fig. 1c).

Commented [ML1]: Discuss Figure 1a

Intercropping significantly increased ~~the~~ bacterial and fungal abundances ~~in both plant species and the fungal abundance~~ in sugarcane. However, no significant difference was observed in the fungi : bacteria (F : B) ratios ~~in the treatments~~ (Fig. 2). Moreover, intercropping increased Chao index of bacteria and fungi; however, significant higher Shannon richness only observed in I-Soybean compared with M-Soybean (Fig. S1d). PCoA analysis showed that the bacterial and fungal communities from different treatment were clearly separated from each other (Fig. S2). ~~The result~~, which indicates crop species and culture mode ~~that~~ influenced the soil microbial community ~~was influenced by crop species and culture mode~~. The dominant phyla, Proteobacteria, Chloroflexi, Acidobacteria, Actinobacteria and Firmicutes for bacteria and Ascomycota, Zygomycota and Basidiomycota for fungi, were the same across treatments (Fig. 3). ~~With the similarities dependent on the crop species and culture mode, the relationships among the treatments were observed in the cluster analyses~~.

~~The~~ CCA analysis (Fig. 4) revealed a relationship between microbial community structure and soil property variables. Significance values for the overall solution and for the CCA1 and

CCA2 axes were 0.035, 0.041, and 0.039 for the bacterial community and 0.005, 0.01, and 0.005 for the fungal community, respectively. The soil pH, SOC, NO_3^-, DOC, MBC, and MBN (p = 0.04, 0.01, 0.03, 0.008, 0.02, and 0.004, respectively, for the bacterial community; p = 0.001, 0.04, 0.04, 0.01, 0.003, and 0.02 for the fungal community) appeared to be strongly correlated with the microbial community.

The X-axis of the PCoA analysis of bacteria and fungi (Fig. S2) was used in the Spearman correlation analysis to detect the relationships between microbial activity and structure (Table 2). A significant relationship was observed between bacterial community and microbial activity; however, the fungal community was not significantly correlated with bacterial communities and microbial activity.

3.3 Effect of intercropping on microbial functional characteristics

Bacterial function predictions were categorized into KEEG pathways. In brief, pathways for nutrient cycles, such as carbon fixation pathways in prokaryotes and citrate cycle (TCA cycle), significant increased, while lipid metabolism, sulfur metabolism and signal transduction mechanisms significant decreased in both I-Sugarcane and I-Soybean (Fig. 5, Table S1). For fungal function, 12 fungal functional guilds, such as wood saprotroph, plant pathogen, plant saprotroph, fungal parasite and endophyte were detected (Fig. 6a). Among the top 5 fungal functional guilds, the relative abundances of wood saprotroph significant increased in I-Sugarcane, and plant pathogen significant increased in ~~I-Sugarcane and~~ I-Soybean~~, respectively~~ (Table S2).

~~Regarding the functional group of wood saprotroph~~From the library generated with ITS

primers, more than 40 OTUs were detected and assigned to the functional group of wood saprotrophs. The top 4 OTUs ~~were~~ belonged to the phylum Ascomycota, with relative abundance ranged from 6.21% to 18.38% (Table S3). Of these, the relative abundances of OTU133 (*Trichoderma*) in I-Sugarcane were significantly higher than M-Sugarcane. Relative abundances of OTU1092 (*Aspergillus*) and OUT126 (*Acremonium*) were significantly higher in both I-Sugarcane and I-Soybean (Fig. 6b). For the functional group of plant pathogen, the top 4 OTUs were belonged to the phylum Ascomycota. Among them, relative abundances of OTU745 (*Gibberella*), OTU1092 (*Clonostachys*) and OUT126 (*Gibellulopsis*) were general lower in intercropping system. Nevertheless, the relative abundance of OTU941 (*Gibellulopsis*) was significantly higher in both I-Sugarcane and I-Soybean (Fig. 6c, Table S4).

4. Discussion

4.1 Effect of intercropping on soil microbial communities

Intercropping of sugarcane with soybean improved microbial properties of microbial respiration, bacterial and fungal abundances and diversity, which is consistent with several previous studies (Sun et al., 2009; Kaur et al., 2000; Li et al, 2016). ~~These results may be attributed to the d~~This may reflect the direct contact of crop roots in the intercropping system~~,~~ which~~therefore stimulating~~ stimulates ~~the~~ roots to release more nutrients ~~for the microbes~~ (Song et al., 2007). Environmental factors, such as pH and SOC, often play important roles in shaping microbial community composition and diversity (Hartman et al., 2008; Tripathi et al., 2018; Ma et al., 2018). Soil pH is a major factor in shifting microbial diversity. The occurrence of this phenomenon may be because the soil pH impacts the acid-base equilibrium of microbial cells

or regulates the availability of soil nutrients (Zhalnina et al. 2015). In this study, soil pH in intercropped soybean soils decreased compared with that in monoculture soil. Additionally, the result of CCA also showed that pH strongly correlated with (p = 0.04 and p = 0.001, for the bacterial and fungal community, respectively) the microbial community. Together, our data ~~indicating~~ indicates that pH ~~was an important factor that~~ governed the microbial community.

SOC ~~has~~ can also ~~been found to be the most important factor that~~ determine~~s~~ microbial community structure in natural environments (Sul et al., 2013; Ma et al., 2018)~~.~~ as~~.~~ increased SOC ~~might provide~~favors ~~copiotrophic~~ copiotrophes ~~microbial community with more favorable conditions than oligotrophic group, and therefore shifted the relative abundance and structure of key microbial group~~ (Fierer et al., 2012; Waring et al., 2013; Luo et al., 2018). In this study, the significant increased SOC in intercropping system and the significant correlation (p = 0.01 and p = 0.04, for the bacterial and fungal community, respectively) between SOC and microbial community (Table 1 and Fig. 4) indicate that SOC ~~also play an important role in shaping~~ shaped the structure of microbial community. ~~In addition to pH and SOC, other soil physiochemical~~physicochemical ~~properties such as~~ NO_3^-, DOC, MBC, and MBN may also have significant impact on the shift of microbial community structure (Fig. 4). Shifting nutrients ~~is known to cause a~~ changes ~~of~~ microbial communities (Sun et al., 2015; Ramirez et al., 2010), since increases in available substrates might increase the activity of ~~cause the copiotrophic~~ copiotrophs ~~group more active~~ in soil (Fierer et al., 2012).

4.2. Effect of intercropping on microbial activity

Carbon-use efficiency, which can be calculated as $C_{growth}/(C_{growth} + C_{respiration})$ has a positive

correlation with microorganism growth and a negative correlation with respiration rates (Spohn et al., 2016). The increased microbial abundance and no significant changed available N in this study may cause an increased carbon-use efficiency and not much change in nitrogen-use efficiency. Therefore, the increased ratios of carbon- and nitrogen-use efficiency in this study may be caused by carbon-use efficiency. However, respiration and the ratios of carbon- and nitrogen-use efficiency, which represent the microbial activity, both increased in the intercropping system in this research. This may be correlated with the nonlinear relationship between microbial growth and respiration (Sinsabaugh et al., 2013). There have many other factors that affect the carbon-use efficiency such as environmental constraints and resource (Sinsabaugh et al., 2013).

~~Higher respiration rates led to lower ratios of carbon- and nitrogen-use efficiency, as carbon-use efficiency can be calculated as $C_{growth}/(C_{growth} + C_{respiration})$ (Spohn et al., 2016). However, respiration and the ratios of carbon- and nitrogen-use efficiency, which represent the microbial activity, both increased in the intercropping system in this research. This may be correlated with available N, which was generally higher in the intercropping treatments, although no statistical significance was detected. This explanation is consistent with the Mooshammer's research, who observed that the carbon- and nitrogen-use efficiency of microorganisms can be adjusted by themselves. The adjustment is dependent on the nutritional availability that low nitrogen-use efficiency and high carbon-use efficiency more likely to appear in the high N availability environment (Mooshammer et al., 2014).~~

Carbon-use efficiency is positively correlated with nutrient availability. Bacterial carbon-use

efficiency tends to increase more markedly with nutrient availability than that with fungi (Keiblinger et al., 2010), as bacteria and fungi usually be considered as copiotrophic (r-selected) and oligotrophic (K-selected) group (Geyer et al., 2016). Additionally, the ratio of fungi and bacteria might shift soil microbial community carbon-use efficiency (Sinsabaugh et al., 2013). In our research, the abundance of bacteria and fungi were significant higher in the ~~intercropping system, although no statistical significance was detected in I-Sugarcane~~I-Soybean system. However, the fungi : bacteria ratio ~~has no~~did not ~~significant difference~~ differ among treatments. Together, more bacteria and fungi abundance and the improved nutrient availability, such as SOC and DOC, in intercropping system may lead to an increase carbon-use efficiency, which subsequently increased the ratios of carbon-use efficiency and nitrogen-use efficiency. Additionally, better nutrient availability~~conditions~~ in the plant-soil-microbial system could improve the plant growth. ~~and~~ The subsequent~~ly~~ release of exudates increase~~s~~ ~~the~~ soil microbial activity~~, for example, via releases of root exudates into the soil, and this subject is worth further exploring~~ (Zhong et al., 2015; Levy et al., 2018).

4.3. The relationship between microbial activity and microbial structure

The changed microbial community structure may result in increased microbial activity in the intercropping system. Analysing dozens of species soil samples from a wide range of ecosystems across America, Fierer et al. (2007) generalized that an appropriate predictor of bacterial phyla abundances was the C mineralization rate. Thus, we assumed that the changed microbial activity may be caused by the shift in the structure of microorganisms. The correlation between the microbial activity and community structure showed that the bacterial community

was significantly correlated with ratios of carbon- and nitrogen-use efficiency and respiration. However, this significant correlation was not observed with the fungal community, which may be attributed to bacteria were hundred times more abundance than fungi (Fig. 2). Therefore, compared to bacteria, the changed fungal community might have a less effect on microbial activity. Nevertheless, the dormancy rates of bacteria are generally higher than fungi (Jones and Lennon 2010). When the dormancy rate reaches a certain level, the quantitative advantage of bacteria cannot explain the high microbial activity and this requires further verification and research. In this study, pH, NO_3^-, MBN, and SOC play important roles in the change in community structure. Our findings indicate that intercropping altered the availability of carbon ~~and nitrogen~~ in the soil. The changed nutrient subsequently allowed bacteria to affect the carbon- and nitrogen-use efficiency of the microorganisms.

4.4. Effect of intercropping on soil functional microorganisms

In general, the prediction of functional microorganisms matched well with our expectations. Functions such as carbon fixation pathways in prokaryotes, citrate cycle (TCA cycle) of bacteria and wood saprotrophs of fungi were found higher in the intercropping system. In contrast, plant pathogens were slightly lower in the intercropping system. The result indicated that the overrepresented functions in intercropping system potentially leading to an accumulation of metabolic products and nutrients. For example, the increased carbon fixation pathways in prokaryotes and citrate cycle (TCA cycle) suggested an acceleration of nutrient conversion (Shi et al. 2017), which might be trigged by the increased microbial activity. Furthermore, the increased OTU133 (*Trichoderma*) belong to wood saprotrophs in intercropped sugarcane may

control a wide range of phytopathogens because *Trichoderma* secretes chitinases and cellulases, which can hydrolyse pathogen cell walls (Bae et al., 2015).

5. Conclusions

In conclusion, sugarcane-soybean intercropping in acidic soil increased microbial diversity and shift soil microbial communities which caused by soil ~~physiochemical~~physicochemical properties (pH, SOC, NO_3^-, DOC, MBC and MBN). The changed ~~microbial~~ bacterial community ~~was as significant~~ correlated to ~~microbial activity was reflected in~~ higher soil respiration rates and nutrient use efficiency ~~in the intercropping system~~. Furthermore, intercropping influenced microbial functions, such as carbon fixation pathways in prokaryotes, citrate cycle (TCA cycle) of bacteria and wood saprotrophs of fungi. These overrepresented functions may ~~acceleration of~~accelerate nutrient conversion and control phytopathogens in soil.

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