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Arbuscular mycorrhizal fungi in roots and soil respond differently to biotic and abiotic factors in the Serengeti

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Abstract

This study explores the relationships of AM fungal abundance and diversity with biotic (host plant, ungulate grazing) and abiotic (soil properties, precipitation) factors in the Serengeti National Park, Tanzania. Soil and root samples were collected from grazed and ungrazed plots at seven sites across steep

had more AM fungal indicator species associated with biotic factors (host plant species and grazing), and soil samples had more indicator species associated with particular sample sites. These findings suggest that regional edaphic conditions shape the site-level species pool from which plant species actively select root-colonizing fungal assemblages modified by grazing. Combining multiple measurements of AM fungal abundance and community composition provides the most informed assessment of the structure of mycorrhizal fungal communities in natural ecosystems.

Introduction

Arbuscular mycorrhizal (AM) fungi are among the most common nutritional symbionts on Earth, yet remarkably little is known about their ecology in natural ecosystems. Recent studies have revealed low global endemism among AM fungi with high local spatial turnover that increases with distance (Davison

regional-scale distribution of AM fungi (Hazard et al. 2013; Rodríguez-Echeverría et al. 2017; Řezáčová et al. 2016). Additionally, biotic variables such as host plant species and host defoliation by herbivores influence AM fungal communities (Murray et al. 2010). Herbivory can reduce the biomass and richness of mycorrhizal fungi by reducing the availability of plant-provided photosynthate (Gehring and Whitham 1994; Kusakabe et al. 2018; Cavagnaro et al. 2018). In contrast, grazing can indirectly stimulate AM biomass through increased dependency on mycorrhizal symbioses to satisfy increased nutrient demands associated with compensatory plant growth (McNaughton 1979; Bardgett et al. 1998; van der Heyde et al. 2019).

The various approaches to identify and quantify AM fungi are imperfect proxies of AM fungal communities because the biomass of AM fungi is allocated differentially inside and outside roots. The composition of AM fungal communities observed in

nutrients while vesicles function as fungal storage units (Smith and Read 2008; Lekberg et al. 2010). Consequently, these structures are likely to be sensitive to factors such as host plant species and defoliation because they are directly involved with the exchange of resources between symbionts. On the other hand, extraradical hyphae may be most sensitive to abiotic factors that influence the availability and mobility of nutrients such as soil texture, pH, mineral composition, and precipitation. Extraradical spores are indicators of reproductive output and dormancy, and their production is likely to be sensitive to host plant responses to disturbance (such as grazing) and seasonal variation in the environment (Bever et al. 2001; Murray et al. 2010; Cuenca and Lovera 2010; Aguilar-Trigueros et al. 2019).

To gain a deeper understanding of the factors that structure AM fungal communities, we studied multiple proxies of abundance and community

intraradical AM fungi is most strongly influenced by biotic factors (grazing and host plant species), and extraradical AM fungi are most strongly influenced by abiotic factors (edaphic and climatic variables).

Materials and methods

Sites

In 1999, a long-term grazing experiment was established in the Serengeti National Park, Tanzania in which six plots (4 × 4 m) were established at each of seven sites spanning 97.4 km between the most distant sites (Table 1; Anderson et al. 2007). At each site, three pairs of plots in closest proximity were randomly assigned to be grazed normally or fenced with 2-m-tall chain-link that effectively excludes all ungulate herbivores (primarily wildebeest, zebras, Thomson's gazelles, buffalo, and topi; McNaughton 1985). The Ngorogoro Volcanic Highland, to the southeast of the Serengeti National Park, creates a

Table 1 Locations and mean annual precipitation (MAP) of the seven study sites in the Serengeti National Park, Tanzania

Data collection

Soil and roots were sampled in May 2012 (Supporting Information Fig. S1). Roots of two dominant C_4 grass species, *Digitaria macroblephara* and *Themeda triandra*, were collected from each plot (n = 84, 7 sites × 2 plant species × 6 plots) and composite soil samples were collected from the holes (approximately 15 cm deep) created by digging up the plants (n = 42). *Themeda triandra* was not present at Barafu, so *Sporobolus fimbriatus* was sampled instead. Within 6 h of collection, roots and soils were air dried for 48 h in a solar drier. After 2 weeks, the dry samples were brought to the laboratory and frozen for long-term storage.

Soil analyses

of Sokoine University of Agriculture in Morogoro, Tanzania (Klute 1986). To measure total phosphorus, calcium, and iron concentrations, 0.3 g subsamples were ground and digested in 7 mL concentrated nitric acid and 3 mL 30% hydrogen peroxide in a Milestone 900 Microwave Digestor (Ethos Inc., Bristol, United Kingdom). Samples were digested for 20 min and reached a maximum temperature of 425 °C. Total soil phosphorus was converted to orthophosphate and then quantified via colorimetry (Grimshaw 1987) on a QuikChem 8000 Series FIA+ (Lachat Instruments, Milwaukee, WI 53218) using QuikChem Method 10–115–01-1-A. Total iron and calcium were measured on an AAnalyst 100 atomic absorption spectrophotometer (Perkin Elmer, Waltham, MA 02451). Samples were compared to in-house standards and external standards produced by Ricca Chemical Company (Arlington, TX 76012) and Hach Company (Loveland, CO 80539).

Percent sand, silt, and clay were determined using

organic matter, total phosphorus, nitrogen, calcium, and iron were multiplied by bulk density to convert values into grams per volume.

Neutral lipid fatty acid analysis

Frozen soil samples were freeze-dried, finely ground with a mortar and pestle, and 5 g of each sample was mixed with a phosphate buffer, methanol, and chloroform. The soil-solvent mixture was separated by centrifugation and then decanted with a 1:2 mix of chloroform and methanol. Phosphate buffer was added and left for phase separation to occur overnight, then the chloroform layer containing the lipids was recovered and reduced by nitrogen flow at 50 °C. Lipids were separated into neutral lipids, glycolipids, and phospholipids by solid-phase extraction by eluting with chloroform, acetone, and methanol, respectively. Lipids were hydrolyzed and methylated. The methylated fatty acids were extracted with hexane and evaporated under

Molecular analysis

DNA was extracted from 70 mg of dried roots from each plant individual (sample) with PowerSoil-htpTM 96 Well Soil DNA Isolation Kit (MO BIO Laboratories, Inc., Carlsbad, CA, USA). The same root DNA extracts were used as in Davison et al. (2015). The following modifications were made to the manufacturer's protocol, following Saks et al. (2013): (1) roots were milled to powder in 2 ml tubes with one or two 3 mm tungsten carbide beads with Mixer Mill MM400 (Retsch GmbH, Haan, Germany) per tube, instead of milling in the Bead Plate; 750 µL of Bead Solution was added to the tubes, mixed, and the slurry transferred to the Bead Plate; (2) to increase DNA yield, Bead Plates were shaken at a higher temperature (60 °C) than in the default protocol—this is suggested by the manufacturer as a variation in order to increase the yield—for 10 min at 150 rpm in a shaking incubator; (3) in order to increase DNA yield but maintain DNA concentration, final elution was performed twice with

GAACCCAAACACTTTGGTTTCC-3′) for the small subunit (SSU) rRNA gene (Lee et al. 2008; Dumbrell et al. 2011). These primers amplify the SSU rRNA gene region, which is taxonomically informative for AM fungi (Öpik et al. 2010; Řezáčová et al. 2016; Vasar et al. 2017). The first PCR was conducted in 8 µL volumes containing 50 nM of each primer, 3.0 mM of $MgCl_2$, 200 µM each dNTP (Phenix Research, Candler, NC), 0.01 U/µL Phusion HotStart II DNA Polymerase (Life Technologies), 1× HF Phusion Buffer (Life Technologies), 6% glycerol, and 1 µL of diluted template DNA. PCR cycling conditions were 95 °C for 2 min followed by 30 cycles of 95 °C for 1 min, 54 °C for 1 min, and 72 °C for 1 min with a final extension at 72 °C for 10 min. Triplicate PCR products were combined by sample, and 2 µL was used to check for results on a 1% agarose gel. The SSU rDNA product was purified using a × 0.8 solution of polyethylene glycol and carboxylated magnetic beads to remove a ~ 100 bp artifact (Rohland and Reich 2012). The

carboxylated magnetic bead solution, quantified, and combined into a final sample library. The library was purified, concentrated, and quantified using quantitative PCR against Illumina DNA standards (Kapa Biosystems, Wilmington, MA). Sequencing was carried out on an Illumina MiSeq System (Illumina Inc., San Diego, CA) running in paired end 2 × 300 bp mode.

Spore analysis

Spores of AM fungi were extracted from approximately 25 g of soil using the wet sieving, sucrose centrifugation method described in Johnson et al. (1999) and McKenney and Lindsey (1987). The diameter of many spores was less than 45 µm, so a sieve with a 25-µm mesh was used on the bottom and a 250-µm mesh was used on the top. All material collected on the top sieve was examined using a dissecting microscope, and large spores and sporocarps were mounted in polyvinyl alcohol

subsamples were oven dried to precisely calculate spore counts per g dry soil. Voucher slides and photographs are maintained at Northern Arizona University.

Data analysis

A total of 20 samples were removed due to low sequence output or because roots were from *Sporobolus fimbriatus* rather than *Themeda triandra* at Barafu. For the remaining 106 samples, OTU picking and taxonomy assignments were performed using the otu_picking_workflow.sh command in akutils v1.2 (Andrews 2018; https://github.com/alk224/akutils-v1.2). Forward reads were trimmed to 250 bp to remove low-quality tails. Reverse sequences were not included in this analysis because they had lower quality than the forward reads and were not joined because of poor quality in the region used for joining reads (Vasar et al. 2017). Demultiplexing was carried out using a

less than 1000 sequences were removed from further analyses. For the remaining 103 samples, taxonomy was assigned to sequences using BLAST, with 90% similarity and an E-value less than 10^{-4}, against the online Maarj*AM* database (http://maarjam.botany.ut.ee; version 04/02/2015; accessed 15 December 2015). Maarj*AM* is a manually curated database containing Glomermycotinan high-quality SSU rDNA sequence data. SSU sequences in Maarj*AM* are phylogenetically clustered into "virtual taxa" (VT) for a standardized taxonomy of Glomeromycotina (Öpik et al. 2010). OTUs not assigned to Glomeromycotina (3.5% of total SSU sequences) were removed before further analyses. All sequences within de novo OTUs that matched the same VT were added together for alpha diversity analyses. Raw sequencing data for samples used in this study are publicly available in the NCBI Sequence Read Archive (study accession SRP158287; BioProject PRJNA486546).

on the Dryad Digital Repository (https://doi.org/10.5061/dryad.2z34tmph7).

Statistical methods

Two-way analysis of variance (ANOVA) was used to test the effects of grazing (fixed) and site (random) on two separate measures of AM fungal abundance (spore density and 16:1ω5c NLFA). In one of our previous analyses, we used model selection of multiple linear regressions with mixed effects to determine that phosphorus was an important predictor of AM fungal abundance using the same NLFA measured here (Stevens et al. 2018). Many of the environmental variables were highly correlated, so to reduce collinearity, a principal component analysis (PCA) was performed on soil pH, iron, calcium, sand, silt, and clay using the "prcomp" function in R (version 3.3.0; R Core Team 2017). Phosphorus was not included in the PCA because, in addition to our previous analyses, a factorial

ash deposits from the Ngorongoro highlands on soil characteristics (clay = 0.45 PCA loading, silt = 0.41, sand = − 0.43, iron = 0.43, calcium = 0.40). The second axis (PC2) explained 17.4% of the variation and was largely influenced by pH (− 0.72 PCA loading), silt (0.43), sand (− 0.33), and calcium (− 0.40). A correlogram was created to visualize relationships between environmental variables, PC1, and PC2 (Fig. S2). All figures were generated using python 2.7 and matplotlib 3.1.0 (Hunter 2007).

Relationships between AM fungal communities and environmental variables were examined using a permutation-based multivariate analysis of variance (PERMANOVA) from the scikit-bio python package version 0.4.2 (http://scikit-bio.org/). Homogenous dispersion of variances was determined using "betadisper" from the "vegan" package (version 2.5–4) in R (version 3.3.0). There was a significant difference in multivariate dispersion among soil and roots (betatdisper $F_{1,101} = 5.81$, $p = 0.017$). Distances

abundances of genera. To summarize overall patterns between AM fungal community composition and abiotic factors, we used the Spearman's rank correlation coefficient in the BIOENV function from scikit-bio. To test the relationship between abiotic characteristics and AM fungal community composition, we used a distance-based redundancy analysis (db-RDA) (Legendre and Anderson 1999) performed with forward and backward model selection using the "capscale" and "ordistep" functions in vegan package version 2.5–4 Oksanen et al. 2018). We performed the db-RDA analysis on Bray-Curtis dissimilarity with default parameters. Indicator species analyses of VT were used to determine which AM fungi were associated with grazing treatments, plant species (for root data only), and sites using the "multipatt" function in the "indicspecies" package (version 1.7.6; De Caceres and Legendre 2009) in R (version 3.3.0) with default values.

highest at BRS (16.2%) and lowest at BAL and TOG, with 6.9% and 7.2%, respectively (Table 2). Total soil nitrogen concentration varied little across the sites (Table 2). Soil phosphorus concentration was highly correlated with calcium ($r^2 = 0.97$) and iron ($r^2 = 0.94$) concentrations. Soil at MSB, BRS, and SOT was mostly silty (58.7%, 55.2%, and 51.8%), while all other sites were sandy (between 47.3 and 67.2%; Table 2).

Table 2 Edaphic properties of the seven study sites with site codes defined in Table 1. Values followed by the same letter do not differ significantly according to Tukey's honestly significant difference test, $p < 0.05$. Values in parentheses are standard deviations. *SOM* soil organic matter (Stevens et al. 2018). For soil texture and calcium and iron concentrations see Dryad Digital Repository (https://doi.org/10.5061/dryad.2z34tmph7)

respectively). Grazing reduced both 16:1ω5c NLFA concentration ($F_{2,28}$ = 3.04; p = 0.09) and spore density ($F_{2,28}$ = 5.63; p = 0.02). There was no interaction between site and grazing for either of the proxies of AM fungal abundance.

Fig. 1

spore density and **c**, **d** the biomarker neutral lipid fatty acid (NLFA; nmol g^{-1}) 16:1ω5c. Shaded regions represent a 95% confidence interval. For all panels, marker color indicates site (abbreviations detailed in Table 1) and shapes indicate grazed (circle) or ungrazed (square) plots

Table 3 Relative abundance, frequency, and abundance g^{-1} soil of arbuscular mycorrhizal fungal spores and auxiliary cell morphotypes by site (codes in Table 1). Relative abundance was calculated as percent of overall total spores. Frequency was calculated as the percent of all plots where a species was present. Standard deviation of spore abundance g^{-1} soil at each site is reported in parentheses

AM fungal diversity based on DNA sequences and spore morphology

($F_{1,101}$ = 19.2, p < 0.001) as visually represented in a PCoA plot (Fig. 2b).

Fig. 2

arranged by decreasing mean annual precipitation (see Table 1 for site codes). Error bars represent a bootstrapped 95% confidence interval. **b** Beta diversity (Bray-Curtis dissimilarity index) of AM fungal VT visualized with a principal coordinate analysis. Blue symbols represent soil AM fungal VT, and green symbols represent root AM fungal VT. A statistically significant difference between soil and root communities was indicated by a PERMANOVA. **c** Relative abundance of AM fungal genera for each community diversity measure: DNA-based diversity in root and soil samples and spore-based morphospecies diversity in soil samples. Genera with abundance below 5% in all three communities are combined in the "Other" category. Genus names that were contemporary with the most recent MaarjAM database from 2015 were used

42 soil samples with an average of 16.9 (± 2.4) morphospecies per sample (Fig. 2a; Table 3). Seven of the 16 AM fungal genera identified according to spore morphology also were identified by DNA sequencing (all seven were found in both soil and roots). All three identification methods (root AM fungal DNA sequencing, soil AM fungal DNA sequencing, and spore morphospecies identification) indicate that the genus *Glomus* dominated the AM fungal community (Fig. 2c). Relative abundances of *Glomus* were 53.5% for soil VT, 51.6% for root VT, and 76.0% for spore morphospecies. *Acaulospora*, *Archaeospora*, *Claroideoglomus*, *Diversispora*, *Paraglomus*, and *Scutellospora* were identified in all three communities (Fig. 2c; Table 3). All genera identified using DNA sequencing also were present as spore morphospecies, but they had different relative abundances according to the two methods (Fig. 2; Table 3).

Predictors of AM fungal community

concentration for root DNA community (adjusted R^2 = 1.7%); silt and sand for soil DNA community (adjusted R^2 = 3.5%); and clay, silt, rainfall, and iron concentration for the spore community (adjusted R^2 = 27.5%) (Fig. 4). There were significant indicator species for site, grazing, and plant species observed in the AM fungal communities assessed using DNA sequencing, but not for the communities assessed using spore morphospecies (Table 4). In soil, 17 VT from six families indicated particular sites. In roots, four VT were indicators of grazing, three VT were indicators of plant host, three VT were indicators of grazed *T. triandra*, and five VT indicated particular sites (Table 4). Only VT 281 (*Paraglomus laccatum*) was an indicator for both soil and roots at TOG; otherwise, there was no overlap in the indicator species for AM fungal communities identified from DNA extracted from soil and roots.

Fig. 3

Curtis dissimilarity. Color bars to the right of the figure represent the range in values of each predictor variable. Spearman rank correlation coefficients (Rho) between AM fungal community composition distance matrices and soil phosphorus concentration (mg cm^{-3}), mean annual precipitation (mm), concentration of the neutral lipid fatty acid NLFA 16:1ω5c in soil (a biomarker for AM fungi; nmol g^{-1}), and soil pH were calculated using BIOENV. For each column, the abscissa is the first principal coordinate axis (percentage variation explained is provided at the bottom of the panels). For each row, the vertical axis is the second principal coordinate axis. The percentage variation explained by the second axis is 9.4% (root), 11.5% (soil), and 13.2% (spore)

Fig. 4

Results for model selection of distance-based redundancy analysis (db-RDA). Significant variables for **a** root DNA, **b** soil DNA, and **c** spore communities were

of the indicator value (func = "IndVal.g") was used as the test statistic. *VT* virtual taxa, *Themeda Themeda triandra*. For sites codes, see Table 1

Discussion

Tropical grasslands harbor diverse, yet understudied AM fungal assemblages (Pärtel et al. 2017; Rodríguez-Echeverría et al. 2017). The Serengeti National Park in Tanzania provides an ideal site to explore the distribution of AM fungi across environmental gradients because they have evolved with the ecosystem for millions of years with minimal human disturbance (McNaughton 1985). Our findings demonstrate that different measures of AM fungal diversity and abundance are correlated with different environmental factors. Specifically, AM fungal indicator species inside plant roots were primarily structured by biotic factors (plant host identity and grazing; Table 4), while communities of AM fungi in

diversity is structured by the biotic and abiotic environment.

Abundance of AM fungi is related to soil phosphorus and precipitation

We observed extremely high densities of AM fungal spores (up to 1210 spores g^{-1} soil) in the Serengeti soils collected during the wet season which was nearly twice the spore density reported from an earlier study of soil collected from the same experimental plots during the dry season (up to 725 spores g^{-1} soil; Antoninka et al. 2015). Both spore density and concentration of NLFA 16:1ω5c in the soil showed a positive correlation with soil phosphorus concentration and a negative correlation with precipitation (Fig. 1; Supporting Information Fig. S2). The positive correlation between the abundance of phosphorus-rich reproductive spores and extraradical hyphae and soil phosphorus concentration is expected in low-fertility, tropical soil and supports

that AM fungal biomass is likely to decline at higher soil phosphorus levels than are observed in the current study (Verbruggen et al. 2013). Inverse gradients of precipitation and soil phosphorus concentration in this natural experiment make it impossible to uncouple the effects of precipitation from the effects of soil phosphorus availability on AM fungal abundance and community structure. However, our natural experiment can inform the design of manipulative experiments. For example, future studies could be designed to experimentally measure the responses of AM fungal hyphal production and decomposition with a full-factorial design of precipitation and phosphorus manipulation treatments.

Grazing and plant host influences mycorrhizal fungi

We observed lower abundance of AM fungal spores and the biomarker NLFA in soil outside the herbivore

belowground nutritional symbionts (Gehring and Whitham 1994; van der Heyde et al. 2019). Furthermore, enrichment of nutrients from the manure of grazing mammals may decrease incentives for plants to invest in AM fungal partners (Bardgett et al. 1998).

We also found that grazing influenced the community composition of intraradical AM fungi (Table 4). Grazing diminishes shading, which has been shown to affect the composition of AM fungal communities (Koorem et al. 2017; Gao et al. 2019). Furthermore, grazing may alter the composition of plant communities, which in turn may influence AM fungal communities. Experimental removal of herbivores in the Serengeti has been shown to decrease plant species richness (Belsky 1992; Anderson et al. 2007). In particular, the cover of *D. macroblephara* increased for 5 years after experimental removal of grazing, while *T. triandra* decreased and eventually disappeared due to its

grazed *T. triandra*; all but one were *Glomus* species (Table 4).

Holistic assessment of AM fungal abundance and diversity

There are potential biases in all proxies of AM fungal abundance and diversity (Sanders 2004), resulting in observed communities deviating from actual communities. This can reflect biological reasons when one considers the contrasting environments for AM fungal growth within roots and in soil surrounding roots, with the intraradical part suggested as "the habitat" for AM fungi (Brundrett 2002; Gao et al. 2019). Methodological causes, however, are multiple. For example, sources of bias in DNA-based analyses stem from sampling strategy, sample size, DNA extraction and amplification methods, and sequence data management (Lindahl et al. 2013; Kohout et al. 2014; Chagnon and Bainard 2015; Hart et al. 2015; Zinger et al. 2019). Spores contain hundreds of nuclei

produce more spores per unit soil than a low biomass of AM fungi (Aguilar-Trigueros et al. 2019), spore biovolume density can be a robust proxy for relative differences in AM fungal abundance across experimental treatments and environmental gradients (e.g., Landis et al. 2004). Combining multiple types of measurements that examine both intra- and extraradical compartments and sampling across multiple seasons and years may be necessary to generate an accurate estimate of AM fungal diversity and community dynamics (Bever et al. 2001; Chagnon and Bainard 2015; Öpik and Davison 2016).

Simultaneous measurement of multiple proxies provides the best estimates of the abundance and species composition of AM fungal communities because different proxies capture different ecological patterns (Chagnon and Bainard 2015). Although most AM fungal VT were identified in both soil and root samples (Fig. 2c), alpha and beta diversity were significantly different (Fig. 2a, b). These differences

structures. A study of local adaptation in grassland AM fungi showed that arbuscule formation was significantly higher when plant genotypes were grown in local soil with their local (co-adapted) AM fungi versus alien counterparts; in contrast, the formation of extraradical hyphae was highest when AM fungal communities were matched with co-adapted soil, but plant genotype identity was irrelevant (Johnson et al. 2010). Clearly, AM fungal structures inside and outside plant roots respond differently to biotic and abiotic stimuli, and AM fungal species allocate biomass differently among root and soil compartments (Hart and Reader 2002). As suggested earlier, plant species may act as filters, selecting AM fungi from a locally available pool of mycorrhizal fungal species (Dumbrell et al. 2010; Chagnon et al. 2012; Bennett et al. 2013; Sepp et al. 2019), which in turn is mostly shaped by the abiotic environment of the habitat (Davison et al. 2016). Communities of AM fungi are dynamic in space and time, and there is no single perfect method to

identified a total of 131 VT from the same DNA extracts from Serengeti root (but not soil) samples with 454 sequencing. Reasons for this difference may include shorter Illumina MiSeq reads compared to 454 reads, or because of stringent quality filtering of sequences resulting in conservative richness estimates. The SSU rRNA gene amplicon used here is relatively robust to short read length, if at least a 180-bp long read is used (Davison et al. 2012; Vasar et al. 2017). Quality filtering of Illumina reads, however, can have a major impact on resultant diversity measures (Vasar et al. 2017; Alberdi et al. 2018; Zinger et al. 2019).

Conclusions

This study demonstrates an effect of the abiotic environment in structuring extraradical AM fungal communities and an effect of biotic factors on intraradical AM fungal communities. A combination of multiple measures of abundance and diversity may

insights gained from studies of intact natural ecosystems such as the Serengeti grasslands may be applied to begin to link community structure with function.

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Conflict of interest

The authors declare that they have no conflict of interest.

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