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Genetic diversity of arbuscular mycorrhizal fungi of *Theobroma cacao* as influenced by environmental factors

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Master's Thesis

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6. Conclusion

This cross-sectional study has provided insights into factors that could influence the diversity of AMF communities in the rhizosphere and roots of cacao trees. The resulting characterization of AMF communities of cacao trees represents a crucial initial step toward the goal of employing these communities in future inoculations to effectively reduce Cd contamination in plants (Schmidt *et al.*, 2022).

The research question of whether differences in the α-diversity indices of AMF communities can be found between two farms has been answered affirmatively. Ten AMF genera were identified on Farm SN, compared to five on Farm CM, resulting in higher AMF richness on Farm SN. Whether soil properties exert the greatest influence on the α-diversity of AMF communities as suggested by Xu *et al.* (2017) cannot be substantiated by this study. However, the analysis of variance from the multiple linear regression models, adjusted to the data of AMF communities in roots and rhizosphere, suggests that soil properties, among other factors, may contribute to differences between the two farms. Zn appears to positively correlate with AMF richness in the rhizosphere, which could be explained by its positive effect on AMF spore and hyphae production, as found in previous studies (Zarei *et al.*, 2008). In this investigation, as in previous research, P demonstrates a negative influence on the AMF community (Oehl *et al.*, 2004; Cheng *et al.*, 2013). This might be attributed to host plants relying less on AMF interactions for adequate phosphorus supply when soil P levels are higher. Fierer and Jackson (2006) identified pH as one of the main drivers of AMF communities. The results of this study also indicate positive correlations between pH and the α-diversity of AMF communities, which may be linked to reduced availability of harmful heavy metals for host plants (Rincón *et al.*, 2021). The observation that CdSoil was positively correlated with all α-diversity indices of AMF communities in the roots was particularly intriguing. In contrast, CdSoil exhibited negative correlations with α-diversity indices of AMF communities in the rhizosphere. Some previous studies have reported negative correlations between heavy metals and the α-diversity of AMF communities, attributing this phenomenon to the detrimental effects of heavy metals on plants, animals, and microorganisms (Yang *et al.*, 2015; Chen *et al.*, 2018b; Genchi *et al.*, 2020). However, these studies did not distinguish between root and rhizosphere compartments. One possible explanation for the positive correlation between CdSoil in the root compartment and α-diversity of AMF communities could be that Cd acts as a kind of species filter (Gucwa-Przepióra and Turnau, 2001; Sánchez-Castro *et al.*, 2017). Due to the heavy metal stress in the investigated soils, cacao trees may recruit beneficial AMF species from the rhizosphere into their roots. This could shape the AMF community towards a community with more (Cd-tolerant) AMF species. Both *Glomus* and *Acaulospora* genera were found at both sites and in both compartments. Previous studies have associated species from these genera with Cd tolerance (Kramadibrata and Herbarium Bogoriense, 2009; Pérez Moncada *et al.*, 2019; Nurhalisyah *et al.*, 2020; Pacheco Flores de Valgaz *et al.*, 2022). Therefore, future experiments should focus on investigating Cd-tolerant species within the *Glomus* and *Acaulospora* genera from both farms. Furthermore, on Farm SN, five AMF genera were identified that were not found on Farm CM namely *Racocetra*, *Septoglomus*, *Redeckera*, *Claroideoglomus*, and *Ambispora*. Additionally, it should be investigated whether the presence of Cd-tolerant *Glomus* and *Acaulospora* species may have facilitated the establishment of other, potentially non-Cd-tolerant species in the roots of *T. cacao*. It could be hypothesized that Cd sequestration within the mycelium of Cd-tolerant AMF species created a more favorable environment for Cd-sensitive species, thereby allowing the colonization of additional AMF species beneficial to cacao trees and contributing to higher α-diversity in roots on Farm SN. Furthermore, climate and management factors, as suggested in previous studies, may also shape AMF communities (Oehl *et al.*, 2004; Ramírez *et al.*, 2016; Bowles *et al.*, 2017; Xu *et al.*, 2017; Rincón *et al.*, 2021; Schmidt *et al.*, 2022). Therefore, future experiments should consider these factors in addition to genotype, grafting method, and tree age. To achieve this, a larger sample size, inclusion of multiple farms and thus climate variables, and standardized management practices are recommended.

The study provides an initial glimpse into soil properties that could shape AMF communities in roots and rhizosphere of *T. cacao*. This sheds some light on characterizing poorly studied AMF communities in tropical regions (Rincón *et al.*, 2021). Further research is needed to uncover the factors influencing AMF communities, with the aim of using them purposefully as inoculants in the future (Verbruggen *et al.*, 2013; Köhl *et al.*, 2016; Droh *et al.*, 2016; Zhang *et al.*, 2019; Medeiros *et al.*, 2023). This could offer an economical method that cacao farmers in South America could employ to specifically reduce Cd uptake in cacao trees, enhance yields and reduce the need of fertilizers or pesticides (Yadav *et al.*, 2017; Zug *et al.*, 2019; Schmidt *et al.*, 2022). Ideally, this would result in less Cd accumulation in cacao beans. Cacao farmers, particularly those producing fine flavored cocoa on plantations with elevated Cd levels, could benefit from this approach. They would be able to maintain the region-specific flavor profile of their cocoa by avoiding blending cacao from various regions to meet Cd limits for export to the EU thus, securing their livelihoods and alleviating poverty (Squicciarini and Swinnen, 2016; Palmer, 2021).