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NOTE

Soil Microbial Diversity Affects the Plant-Root Colonization by Arbuscular Mycorrhizal Fungi

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Abstract

Terrestrial plants establish symbiosis with arbuscular mycorrhizal fungi (AMF) to exchange water and nutrients. However, the extent to which soil biodiversity influences such association remains still unclear. Here, we manipulated the soil microbial diversity using a "dilution-to-extinction" approach in a controlled pot microcosm system and quantified the root length colonization of maize plants by the AMF *Rhizophagus clarus*. The experiment was performed by manipulating the soil microbiome within a native and foreign soil having distinct physicochemical properties. Overall, our data revealed significant positive correlations between the soil microbial diversity and AMF colonization. Most importantly, this finding opposes the diversity-invasibility hypothesis and highlights for a potential overall helper effect of the soil biodiversity on plant-AMF symbiosis.

Keywords Soil biodiversity · Dilution-to-extinction · *Rhizophagus clarus* · Symbiosis · Plant-microbe interaction

Introduction

Arbuscular mycorrhizal fungi (AMF) establish symbiosis with approximately 70–90% of all land plant species [1]. This symbiosis allows for large-scale exploration of the soil matrix through the extensive formation of fungal extraradical mycelium, and results in enhanced uptake of water and nutrients (predominantly phosphate). Most of the studies on plant-AMF association have been focusing on understanding the beneficial effects of AMF for plant growth and enhancement of plant protection against pathogens and abiotic stresses (e.g., [2, 3]). Worth noticing, these studies have largely investigated plant-AMF interaction as a bipartite system. Here, we hypothesized that the soil biodiversity status constitutes an additional factor that can exert a significant and yet unexplored effect on the rate of colonization of plant-roots by AMF. This hypothesis aligns with the holistic notion that biodiversity has a direct influence on ecosystem multifunctionality.

To test this hypothesis, we applied a framework similar to that used to study biodiversity-invasibility effects [4]. In brief, we manipulated the level of soil biodiversity in a pot experiment using the "dilution-to-extinction" approach. For that, two distinct Dutch agricultural soil types were used, named Buinen (B) and Wildekamp (W) (Fig. 1a). While soil B was used as a microbial donor and recipient, soil W was only used as a recipient of microbial dilutions extracted from soil B. This design allowed for testing consistency and reproducibility of the results within a native and foreign transplanted soil microbiome. Prior to inoculation, soils in each pot were gamma-irradiated (50 kGy), and serially diluted soil:water solutions from the original soil B (10^{-1}, 10^{-3}, and 10^{-6}) were added individually per pot ($n = 3$ per treatment) (see [4] for

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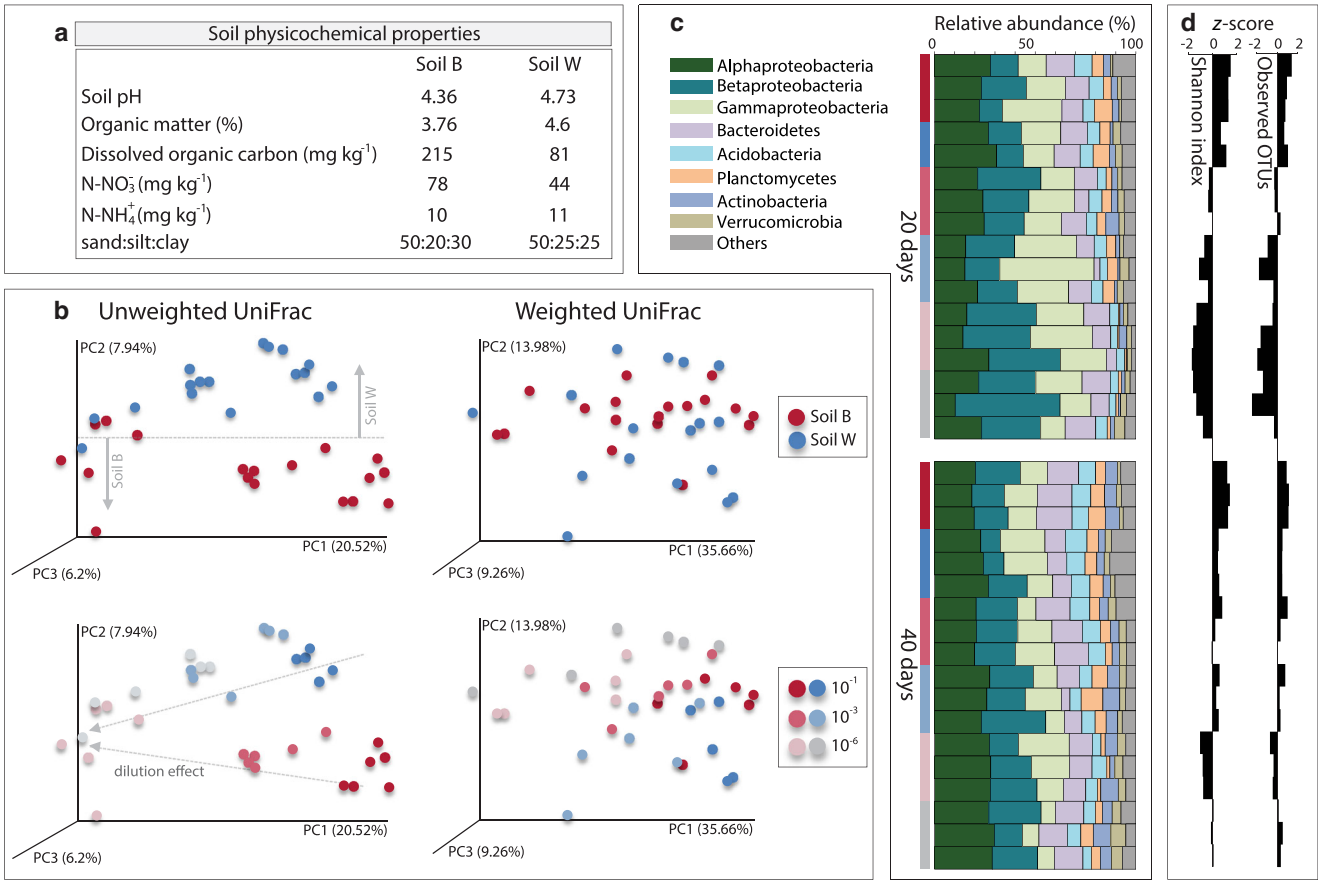

Fig. 1 Alfa- and beta-diversity gradients in soil bacterial communities obtained using the "dilution-to-extinction" approach. **a** Physicochemical properties of the two distinct Dutch agricultural soils used in this study, Buinen (B) and Wildekamp (W). **b** Principal coordinates analysis based on unweighted and weighted UniFrac distances [10]. Different colors indicate distinct soil types and the gradient of each respective color indicates the dilution treatment. **c** Relative abundances (%) of bacterial taxa displayed at the phylum level, except the phylum Proteobacteria (shown at the level of classes). Low abundant groups (i.e., total average < 0.5%) are shown as "others." **d** Total number of Observed OTUs and Shannon index shown as *z*-score transformed data. High-throughput sequencing data were analyzed using the Brazilian Microbiome Project Operating System (BMPOS) [6]. The entire dataset was rarefied to a depth of 2500 sequences per sample (the fewest in a single sample) to minimize effects of sampling effort on the statistical analysis

details). All pots were kept for 30 days at 75% of the water holding capacity to establish comparable and relatively stable soil bacterial abundances (i.e., ~10^7 CFU g^{-1} of soil). After this time, pots were inoculated with 30 spores of *Rhizophagus clarus* and sowed with surface-sterilized maize seeds. Control (non-inoculated) pots were run per each treatment ($n = 3$). The experiment was harvested by collecting the rhizosphere soils after 20 and 40 days. Soil total DNA was extracted using the DNeasy® PowerSoil Kit (QIAGEN), and bacterial communities were profiled using the primer set 515F/926R in an Illumina MiSeq sequencing platform (2 × 250 bp). Sequence data analysis was performed as recommended by the Brazilian Microbiome Project (BMP) [5], using the BMP Operating System [6]. The determination of root length colonization (RLC, %) was carried out by keeping the roots in 10% KOH for 12 h, followed by AMF staining using ink pen solution (0.05%) for 1 min at 90 °C [7–9]. RLC (%) was determined using light microscopy by visually inspecting a total of 100 standardized root fragments per sample.

We first validated the gradient of soil biodiversity in our experimental system by calculating the differences in bacterial community structures in both soils inoculated with dilutions of 10^{-1}, 10^{-3}, and 10^{-6}. Principal coordinates analysis using both weighted and unweighted UniFrac distances [10] revealed significant differences in bacterial communities per soil type across the gradient (PERMANOVA, Pseudo-$F = 2.262$, $P = 0.045$; Pseudo-$F = 2.372$, $P = 0.005$, respectively) (Fig. 1b). These results indicate that despite similar abundant bacterial taxa dominate in both soils, the applied treatments—across the diversity gradient—have significant differences in community composition (Fig. 1c). Considering both soil types (B and W) and sampling times (20 and 40 days) together, the gradient of inoculation resulted in variations of OTU counts ranging from 439 ± 39 to 250 ± 60 OTUs (average ± std.; Tukey's *t* test $P < 0.0001$) in pots inoculated with dilutions of 10^{-1} to 10^{-6}, respectively. Likewise, the Shannon diversity index varied from 7.0 ± 0.25 to 5.6 ± 0.8 (Tukey's *t* test $P < 0.0001$) (Fig. 1d). Collectively, these results support the

significant and gradual effects of the dilution treatments in the soil bacterial community structure and diversity (Fig. 1).

Samples were collected at two time-points (i.e., 20 and 40 days), both of which displayed consistencies with respect to similar plant phenotypes within treatments in each time (i.e., similar growth, absence of symptoms related to nutrient deficiency, and absence of disease), indicating reproducibility and lack of potential confounding effects. Quantification of RLC (%) at day 20 ranged from 43 ± 15 and 41 ± 15 for soils B and W, respectively, whereas values at day 40 were significantly lower (36 ± 4 and 34 ± 4 for soils B and W, respectively, Supplementary Table S1) (Tukey's *t* test $P = 0.009$). This likely occurred due to the larger root volume in plants collected at day 40. Of key importance, no AMF colonization was detected in the control pots (i.e., non-inoculated pots at both 20 and 40 days). Most interestingly, by taking together both soil (B and W) gradients, the level of soil diversity (i.e., total number of bacterial OTUs and Shannon index) significantly correlated with RLC (%) at day 20 ($R^2 = 0.653$, $P < 0.0001$, and $R^2 = 0.408$, $P = 0.0056$, respectively) (Fig. 2a, b). However, such correlations were not found to be significant at day 40 ($P > 0.05$, Fig. 2 c, d).

The literature highlights that biodiversity can operate as a buffer against alien species invasion attempts, known as the "diversity-invasibility hypothesis" (DIH) [11, 12]. Importantly, this hypothesis does not distinguish between different organismal lifestyles (i.e., biotrophs, autotrophs, or heterotrophs) and, in most cases, evokes the concepts of niche occupancy, ecological competition, and niche displacement as potential mechanisms underpinning the phenomenon [4]. Thus, it is possible to envision that beneficial types of ecological association between microbial taxa with distinct lifestyles can occur, which might counter the DIH. In the particular case of plant-AMF colonization, it has been reported that several bacterial taxa can exert beneficial and synergistic effects with AMF [13], the so-called mycorrhiza helper bacteria [14]. For example, bacteria belonging to the Oxalobacteraceae family have been reported to preferentially associate with mycorrhizal roots [15, 16]. Also, members within this family were shown to promote AMF spore germination, hyphal growth, and root colonization of *Glomus mosseae* [17] (also see [13, 18, 19]).

Here, we used a more holistic approach based on whole community diversity manipulation. In doing so, we found the correlation between RLC and soil biodiversity to be time-dependent, i.e., significant at day 20 but not at day 40. This indicates that once plants establish in the soil, the potential beneficial association between specific bacterial taxa (or the overall biodiversity) and AMF exerts a minor or non-significant effect on AMF colonization rate. We acknowledge that this finding is still preliminary, and advocate the need for further studies aiming at properly partitioning this evidenced time-dependency. Besides, the biodiversity-AMF colonization relationship remains still to be tested across distinct environmental contexts, i.e., plant species and growth developmental stages; plant-AMF species specificity [20]; and soil physicochemical and biological characteristics (e.g., pH, nutrient content). As such, caution is warranted in terms of generalizing the overall patterns found in this study. However, from a conceptual point of view, our finding nicely illustrates the importance of time series analysis in studying AMF-plant-soil biodiversity interactions, with direct implication for attempts to enhance or promote AMF manipulation and plant colonization. Last, we posit that a comprehensive understanding of how ecological interactions stimulate ecosystem functioning (e.g., by favoring different resource pool utilization across organisms with differentiation in life strategy) can only be achieved by taking into account the intrinsic status and composition of soil microbiomes in a tripartite microbiome-plant-AMF interactive system. Such conceptual extension

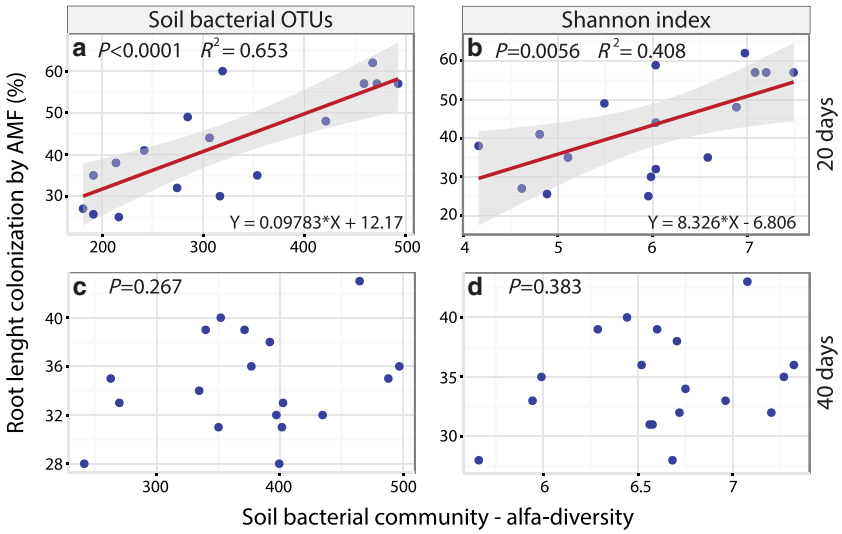

Fig. 2 Linear regression models displaying correlations between alfa-diversity metrics of soil bacterial communities—i.e., **a**, **c** OTU richness and **b**, **d** Shannon diversity index—and the plant root length colonization (RLC, %) by AMF. Data are shown as a function of the progressive increments in alfa-diversity, and correlational plots are separated by sampling time (i.e., 20 and 40 days). The solid lines are linear regression models (contour lines are 95% confidence intervals), and statistics are provided on the panels

corroborates with a more inclusive view of the biodiversity hypothesis [21], and advance knowledge of the distinct facets by which biodiversity promotes ecosystem multifunctionality.

Author Contributions DAF, JFS, and FDA developed the experimental design; DAF and TFS performed the experiment and data collection; VSP and FD-A analyzed the data; FD-A wrote the manuscript with contributions from all authors.

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Data Availability All sequence data and metadata were deposited in the Sequence Read Archive (SRA; http://www.ncbi.nlm.nih.gov/Traces/sra/) under the BioProject PRJNA599204: "Soil microbial diversity affects the plant-root colonization by arbuscular mycorrhizal fungi" (https://www.ncbi.nlm.nih.gov/bioproject/PRJNA599204).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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