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SHORT COMMUNICATION

Are lipids, phenylpropanoids, and benzenoids potential metabolite biomarkers for succession in desert biocrusts?

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Abstract
Metabolites can provide useful biomarkers to indicate changes in the soil microbial community. Here, we quantitatively assessed potential metabolite markers associated with biocrusts from early successional stages (cyanobacteria) to late successional stages (moss or lichens) in a desert ecosystem via untargeted metabolomics analysis. We identified 570 metabolites in total, several of which varied substantially among the different successional stages, thus serving as potential successional-stage biomarkers. Lipids and lipid-like molecules, as well as several volatile organic compounds (phenylpropanoids and polyketides/benzenoids), may serve as potential biomarkers to identify stages of the cyanobacteria-lichen-moss successional trajectory in biocrusts.

Keywords Cyanobacteria · Lichen · Moss · Tengger Desert

Introduction

Biocrusts cover about 12% of Earth's terrestrial landmass (Rodriguez-Caballero et al. 2018) and provide essential ecosystem functions in arid environments (Lan et al. 2011; Li et al. 2021; Veste et al. 2021). Ecological succession (level of development) in biocrusts following external perturbation typically follows a trajectory where soil crusts are initially dominated by early successional cyanobacteria that colonize bare ground, fix nitrogen and aggregate soil, and are eventually replaced by later successional stages dominated by either lichens or mosses (Li 2012; Bowker et al. 2014). Morphological properties of photosynthetic organisms are typically used to distinguish the successional stages of biocrusts (Mallen-Cooper et al. 2019; Machado de Lima et al. 2021). Among the cryptogamic species, cyanobacteria frequently occurred during the initial successional stages of biocrusts. Subsequently, green algae and lichen gradually appeared, while mosses finally colonized in the later stages of biocrusts and there were some combinations or transitions between these stages (Li et al. 2002, 2010). However, the external morphology of taxa in biocrusts is a rough measure of successional stage. Instead, direct measurements of the metabolites formed by the organisms within these biocrusts can not only provide a useful way to identify the successional stages of biocrusts, but can also provide important information on the underlying functional processes within biocrusts and their influence on ecosystem functions as they change in time and space (Li 2012). While bits of metabolite, such as phospholipid fatty acids (PLFAs), have been used in studying specific samples and content variation characters of biocrust under different climate condition (Zaady et al. 2010), the application of metabolite as biomarkers for biocrusts succession has received limited attention in previous reports.

Materials and methods

To identify metabolite biomarkers of different successional stages of biocrusts, we analyzed the untargeted metabolomics of biocrusts at different successional stages, including early successional cyanobacteria, three types of mixed-stage biocrusts at the transition between

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cyanobacteria, lichens and/or mosses, and two distinct late successional stages (lichen and moss). We collected samples on the southeast edge of the Tengger Desert (37°27′ N, 104°46′ E; elevation: 1570 m a.s.l.) in northern China at the end of August 2021. To avoid terrain and vegetation influences on biocrust development, we randomly collected all samples from undisturbed soil in the spaces between shrubs. For each of the six different biocrust types, we collected nine soil cores (3.5 cm diameter and 0.5 cm depth) using a sterile trowel, resulting in a total of 54 samples. To prepare for the metabolomic analysis, we randomly selected three of these biocrust cores and thoroughly mixed them in the field to form a composite sample, as a result, triplicate samples were collected from each biocrust type. Each composite sample was then preserved in an icebox for transportation to the laboratory. The samples were sieved through a 2-mm-diameter sieve and subjected to untargeted metabolomic analysis (Jenkins et al. 2017) using a UHPLC system (Agilent 1290 Infinity II LC System, Agilent Technologies, Santa Clara, CA, USA). Through a search against our in-house tandem mass spectrometry (MS2) database, i.e., the Biotree database (Zhang et al. 2023), we identified 151 metabolites in negative ion modes and 419 metabolites in positive ion modes. We selected metabolites with a MS2 score of over 0.90 (Table S1) for further analysis as differential metabolites. To do so, we screened differential metabolites with $p < 0.05$, and calculated the value of variable significance in projection (VIP) of the first principal component using orthogonal projections to latent structure-discriminate analysis (OPLS-DA). The specific description of each step of study is shown in Supplemental Methods.

Results and discussion

In Fig. 1 and Figure S1, we illustrate differential metabolites that were indicative of differences between successional stages of the biocrusts. Specifically, we found that the top three metabolites that differentiated cyanobacteria crust and cyanobacteria-lichen crust were genistein, 3-hydroxyphenylacetic acid, and *N*-succinyl-LL-26-diaminopimelate. The top three metabolites that differentiated cyanobacteria crust and lichen crust were eicosapentaenoic acid, arachidonic acid, and 6,8-di-O-methylaverufin. The top three metabolites that differentiated cyanobacteria crust and cyanobacteria-moss crust were eicosapentaenoic acid, arachidonic acid, and 6,8-di-O-methylaverufin, and those that differentiated between cyanobacteria crust and moss crust

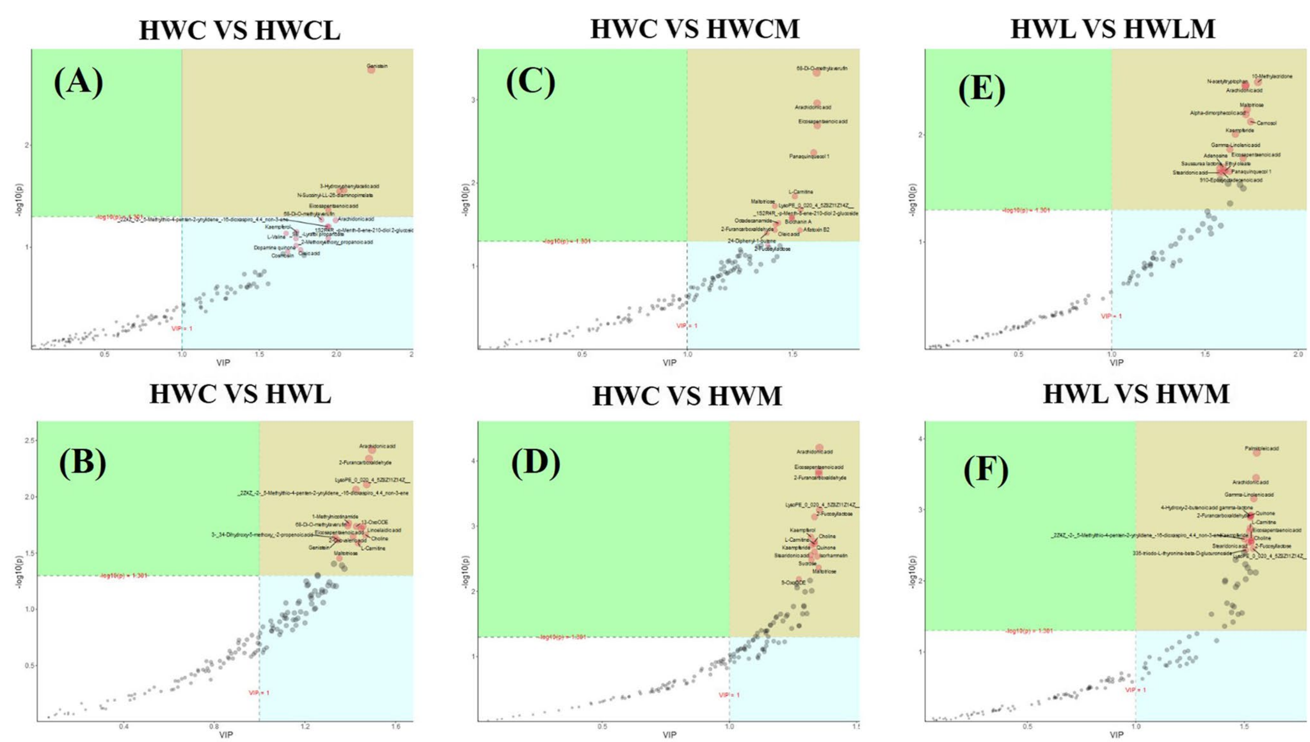

Fig. 1 Orthogonal projections to latent structures-discriminate analysis (OPLS-DA) of differential metabolites among biocrust successional stages. The stages include cyanobacteria (HWC), lichen (HWL), moss (HWM), cyanobacteria-lichen (HWCL), cyanobacteria-moss (HWCM) and lichen-moss (HWLM). (**A**) Differential metabolites between HWC and HWCL; (**B**) Differential metabolites between HWC and HWCM; (**C**) Differential metabolites between HWL and HWLM; (**D**) Differential metabolites between HWC and HWL; (**E**) Differential metabolites between HWC and HWM; (**F**) Differential metabolites between HWL and HWM

were LysoPE(0:0/20:4(5Z,8Z,11Z,14Z)), arachidonic acid, and 2-furancarboxaldehyde. Finally, the top three metabolites that differentiated between moss crust and moss-lichen crust were 10-methylacridone, carnosol, and maltotriose, while those differentiated between moss crust and lichen crust were palmitoleic acid, arachidonic acid, and maltotriose. Among the cyanobacteria to lichen stages, we identified five metabolites that were strongly associated with the successional sequence (Fig. 2A), nine that associated with the successional sequence from cyanobacteria to moss (Fig. 2B), and seven that associated with the successional sequence from lichen to moss (Fig. 2C). For each successional transition, several common differential metabolites significantly increased from early to late successional stages and one (6, 8-di-O-methylaverufin) decreased (Fig. 3; Table S2).

Of the potential metabolite biomarkers that we identified, a large proportion (9/14) were lipids and lipid-like molecules. These metabolites are particularly useful biomarkers because they have high structural diversity and biological specificity, and provide good indicators of microbial biomass, community composition, and function (Zelles 1999; Bååth 2003). The study conducted in Negev Desert revealed significant differences in the total PLFA, which is one of lipid and lipid-like molecules, which is used to determine soil microbial biomass (Zelles 1999; Joergensen 2022), within the biocrusts developed under varying aridity gradients (Zaady et al. 2010). Additionally, the concentration of linoleic acid (one of lipid) showed a positive correlation between the total PLFA-based biomass (Zelles 1999). Zaady et al. (2010) also observed a strong correlation between the total PLFA-based biomass and the polysaccharide content in biocrusts, which increased a long succession pathway (Lan et al. 2011). We hypothesize that these variations in lipid and lipid-like molecules occur due to significant changes in bacterial and fungal species composition and biomass, as well as alterations in polysaccharide content during biocrust succession (Gundlapally and Garcia-Pichel 2006; Lan et al. 2011; Wang et al. 2015; Liu et al. 2017). As a result, these molecules can serve as valuable biomarkers for distinguishing different biocrust successional stages.

The second most differentiated group of metabolites in our study were volatile organic compounds, including phenylpropanoids and polyketides/benzenoids. These metabolites are produced and transformed by soil cyanobacteria and microalgae (e.g., *Lyngbya* spp., *Nostoc* spp.) and provide important ecological functions in soils (Jordan et al. 1993; Van Wagoner et al. 2007; Raj et al. 2014). However, microbial activity in soils is greatly affected by environmental factors (e.g., moisture, pH, temperature) (Jordan et al. 1993) and can change substantially during biocrust succession (Li 2012; Weber et al. 2022). Therefore, caution is required to make these metabolites biomarkers for tracing biocrust succession.

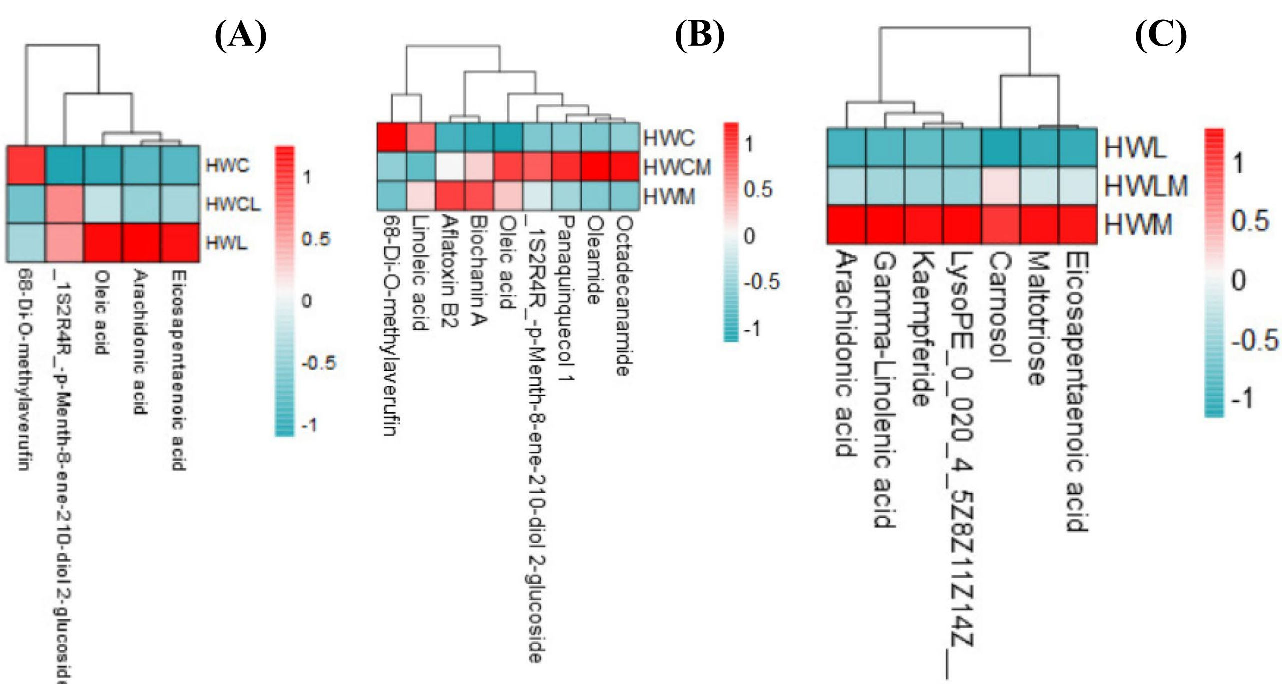

Fig. 2 Heat map showing the distribution patterns of metabolites in biocrusts of different successional stages. **A** Cyanobacteria-lichen successional stage. **B** Cyanobacteria-moss successional stage. **C** Lichen-moss successional stage. Cyanobacteria (HWC), lichen (HWL), moss (HWM), cyanobacteria-lichen (HWCL), cyanobacteria-moss (HWCM) and lichen-moss (HWLM)

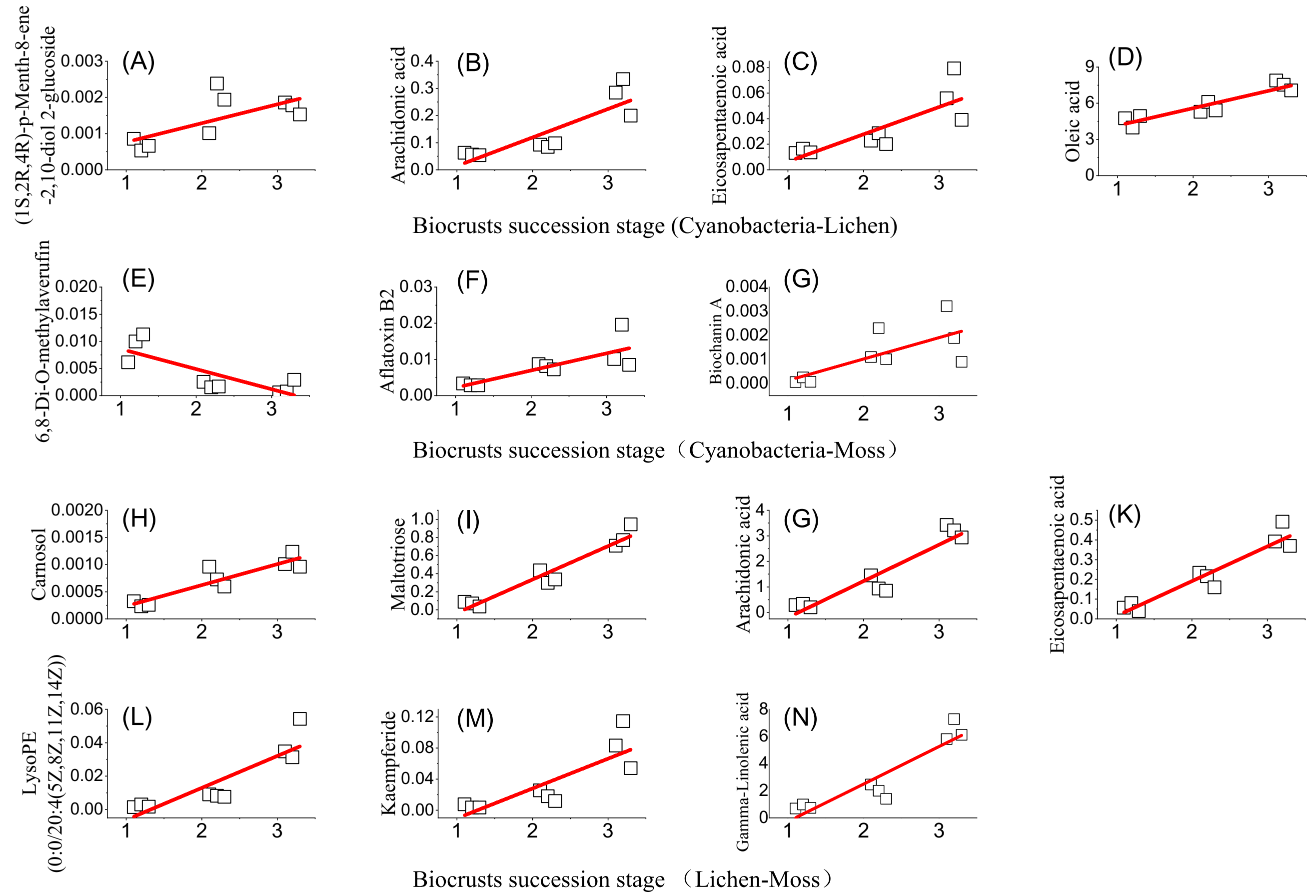

Fig. 3 General linear model showing the relation between biocrust successional stage and different metabolites. Squares represent observed data, and solid lines represent fitted-linear regression. (**A**)-(**D**) Relation between Cyanobacteria-lichen successional stage and (1S,2R,4R)-p-Menth-8-ene-2,10-diol 2-glucoside, Arachidonic acid, Eicosapentaenoic acid and Oleic acid; (**E**)-(**G**) Relation between Cyanobacteria-moss successional stage and 6,8-Di-O-methylaverufin, Aflatoxin B2 and Biochanin A; (**H**)-(**N**) Relation between Lichen-moss and Carnosol, Maltotriose, Arachidonic acid, Eicosapentaenoic acid, LysoPE(0:0/20:4(5Z,8Z,11Z,14Z)), Kaempferide and Gamma-Linolenic acid

Conclusions

In all, we demonstrated that metabolites associated with transitions of microbial community composition differentiate along biocrust successional trajectories. As a result, we suggest that lipids, along with phenylpropanoids and benzenoids, may be suitable as potential indicators for predicting the successional stage of biocrusts. However, caution is essential when phenylpropanoids and benzenoids are used as biomarkers, as those metabolites depend not only on biocrust succession but also on biological activity. Methods for studying the microbiological community at the ecosystem level are flawed because they use samples, whose size is not that required for ecosystem-scale research. As a result, generating compatible microbial and ecosystem data sets has proven challenging. The analysis of soil metabolites such as lipids is promising because it can measure microbial biomass and also conduct subgroup/molecular profiling (Balser et al. 2019). The use of metabolite biomarkers may be a sensitive measurement of biocrusts' successional stage, particularly important for biocrust of desert soils so as to ensure their sustainable use. However, the current results were only obtained from one study site and to generalize these results we need further investigations using several different soils and determining the role of soil microbial communities in affecting the concentrations of these biomarkers.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s00374-023-01767-9.

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Data availability Data will be made available on request.

Declarations

Competing interests The authors declare no competing interests.

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