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Land use as a driver for the structure and function of biological soil crusts in mesic environments

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“Do what you are scared to do and watch your confidence grow.”

Grant Cardone

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Summary

Biological soil crusts have been studied for decades. They are known from (semi-) arid regions and nutrient-poor areas worldwide as pioneer communities consisting of soil particles, phototrophic and heterotrophic organisms. The dominant phototrophic groups are cyanobacteria, algae, mosses, and lichens, increasing carbon contents due to their photosynthetic activity. The properties and structure of biological soil crusts are determined by biological processes associated with the colonization of organisms. Due to the fixation of carbon and nitrogen from the atmosphere, the excretion of extrapolymeric substances, and the filamentous growth of their members, biological soil crusts play crucial roles in nutrient cycling, water infiltration, and soil stabilization. As biological soil crusts mature, nutrient contents increase, attracting heterotrophic organisms and increasing microbial biomass and diversity. Biological soil crusts have also been detected in mesic regions, but their development, functionality, and community compositions have not been studied in detail. Specifically, biological soil crusts from managed, human-impacted areas like forests or agricultural fields are poorly understood. This thesis aimed to understand the influence of microbial members of biological soil crusts from managed, mesic sites on biogeochemical cycles. Thus, it investigated their community composition, structure, and functional potential to influence nutrient cycles and pools.

It was shown that biological soil crusts develop on human-impacted sites of mesic regions within one growing season. Contrary to their counterparts from drylands, where disturbances cause long regeneration times, in mesic regions, biological soil crusts need soil surface disturbances to create bare soil and get a chance to develop. They display the final successional stages in (semi-) arid and nutrient-poor areas. At the same time, in mesic regions, they occur as ephemeral stages after soil surface disturbances until they are covered or shaded or both by developing higher plants. Chlorophyll analysis revealed that photosynthesis mainly takes place in the biological soil crusts. However, as chlorophyll was further detected in small amounts in bare soil samples, it can be assumed that the bare soil is in a pre-crust stage. As known from drylands, mesic biological soil crusts retained nutrients and formed a hotspot for microorganisms. Additionally, the functional potentials for nitrogen and phosphorus turnover and the proportions of copiotrophic microorganisms were higher in biological soil crusts than in bare soils. This was accompanied by a lower diversity, as observed from other hotspots like the rhizosphere. Furthermore, biological soil crusts promoted the interaction of inhabiting microorganisms. Particular copiotrophic taxa profited from the biocrust formation and were highly involved in the networks. This was even enhanced on agricultural fields with organic fertilizer. The network hubs were identified as taxa involved in the degradation of organic material and the production of extrapolymeric substances. This

is why these were assumed to be key characteristics of biocrusts in mesic, managed environments. Notably, sites with lower nutrients or soil quality revealed typical characteristics of biological soil crusts. Nonetheless, biological soil crusts were also detected on nutrient-rich sites. However, the availability of the increased nutrients for plants, the influence of the different nutrient transformations, and investigations about the establishment and the duration over a year need to be analyzed in future studies.

Zusammenfassung

Biologische Bodenkrusten sind aus (semi-)ariden Regionen und nährstoffarmen Gebieten weltweit bekannt und werden seit vielen Jahrzehnten untersucht. Biologische Bodenkrusten sind Pioniergemeinschaften, die aus Bodenpartikeln, phototrophen und heterotrophen Organismen bestehen. Die dominierenden phototrophen Gruppen sind Cyanobakterien, Algen, Moose und Flechten, die durch ihre photosynthetische Aktivität den Kohlenstoffgehalt erhöhen. Die Eigenschaften und die Struktur von biologischen Bodenkrusten werden durch biologische Prozesse bestimmt, die mit der Ansiedlung von Organismen einhergehen. Durch die Fixierung von Kohlenstoff und Stickstoff aus der Atmosphäre, die Ausscheidung extrapolymerer Substanzen und das fadenförmige Wachstum ihrer Mitglieder spielen biologische Bodenkrusten eine entscheidende Rolle im Nährstoffkreislauf, bei der Wasserinfiltration und der Bodenstabilisierung. Wenn biologische Bodenkrusten reifen, steigt der Nährstoffgehalt, was heterotrophe Organismen anlockt und zu einer höheren mikrobiellen Biomasse und Vielfalt führt. Biologische Bodenkrusten wurden auch in mesischen Regionen nachgewiesen, aber ihre Entwicklung, Funktionsweise und Zusammensetzung der Lebensgemeinschaften wurden bisher nicht im Detail untersucht. Insbesondere über biologische Bodenkrusten aus bewirtschafteten, vom Menschen beeinflussten Gebieten wie Wäldern oder landwirtschaftlichen Feldern ist nur wenig bekannt. Ziel dieser Arbeit war es, den Einfluss der mikrobiellen Mitglieder von biologischen Bodenkrusten aus bewirtschafteten, mesischen Standorten auf biogeochemische Kreisläufe zu verstehen und dazu ihre Gemeinschaftszusammensetzung und -struktur sowie ihr funktionelles Potenzial zur Beeinflussung von Nährstoffkreisläufen und -pools zu untersuchen.

Es wurde gezeigt, dass sich biologische Bodenkrusten auf vom Menschen beeinflussten Standorten in mesischen Regionen innerhalb einer Vegetationsperiode entwickeln. Im Gegensatz zu ihren Pendants aus Trockengebieten, wo Störungen lange Regenerationszeiten verursachen, benötigen sie in mesischen Regionen Störungen der Bodenoberfläche, um nackten Boden zu schaffen und eine Chance zur Entwicklung zu erhalten. In (semi-) ariden und nährstoffarmen Gebieten stellen sie die letzten Sukzessionsstadien dar, während sie in mesischen Regionen als Übergangsstadien nach Störungen der Bodenoberfläche auftreten, bis sie von sich entwickelnden höheren Pflanzen bedeckt oder beschattet werden oder beides. Die Chlorophyllanalyse ergab, dass die Photosynthese hauptsächlich in den biologischen Bodenkrusten stattfindet, aber da Chlorophyll auch in Bodenproben nachgewiesen wurde, kann davon ausgegangen werden, dass sich der Boden in einem Vorkrustenstadium befindet. Wie in Trockengebieten, halten mesische biologische Bodenkrusten Nährstoffe zurück und bilden einen Hotspot für Mikroorganismen. Darüber hinaus waren die funktionellen Potenziale für den Stickstoff- und

Phosphorumsatz und der Anteil copiotropher Mikroorganismen in biologischen Bodenkrusten höher als im Boden. Dies ging mit einer geringeren Diversität einher, wie sie auch an anderen Hotspots wie der Rhizosphäre beobachtet wurde. Außerdem förderten biologische Bodenkrusten die Interaktion der dort lebenden Mikroorganismen. Bestimmte copiotrophe Taxa profitierten von der Biokrustenbildung und waren stark an den Netzwerken beteiligt. Dies wurde auf landwirtschaftlichen Flächen mit organischem Dünger noch verstärkt. Als Knotenpunkte des Netzwerks wurden Taxa identifiziert, die am Abbau von organischem Material und an der Produktion extrapolymerer Substanzen beteiligt sind, weshalb davon ausgegangen wurde, dass dies die Hauptmerkmale von Biokrusten in mesischen, bewirtschafteten Standorten sind. Insbesondere auf Flächen mit geringerem Nährstoffgehalt oder geringerer Bodenqualität wurden vermehrt typische Merkmale von biologischen Bodenkrusten festgestellt. Nichtsdestotrotz, biologische Bodenkrusten entwickelten sich auch auf nährstoffreichen Standorten. Die Verfügbarkeit der vermehrten Nährstoffe für Pflanzen oder der Einfluss der verschiedenen Nährstoffumwandlungen sowie Untersuchungen über die Etablierung und die Dauer im Laufe eines Jahres müssen jedoch in zukünftigen Studien analysiert werden.

List of Abbreviations and Acronyms

Alb	Schwäbische Alb
ASVs	Amplicon sequencing variants
ATP	Adenosintriphosphat
BSA	Bovine serum albumin
CTAB	Hexadecyl trimethyl ammonium bromide
DEPC	Diethylpyrocarbonate
DIC	Dissolved inorganic carbon
DOC	Dissolved organic carbon
DON	Dissolved organic nitrogen
EPS	Extrapolymeric substances
Hainich	Hainich-Dün
PCR	Polymerase chain reaction
PEG	Polyethylene glycol
qPCR	Quantitative polymerase chain reaction
Schorfheide	Schorfheide-Chorin

List of Publications and Contributions

- Gall C., Ohan J., Karsten U., Schloter M., Scholten T., Schulz S., Seitz S., Kurth, J. K. (2022). “Biocrusts: Overlooked hotspots of managed soils in mesic environments”. *Journal of Plant Nutrition and Soil Science*, https://doi.org/10.1002/jpln.202200252.
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Further publications

- Grafe, M., Kurth, J. K., Panten, K., Raj, A. D., Baum, C., Zimmer, D., Leinweber, P., Schloter, M., & Schulz, S. (2021). „Effects of different innovative bone char based P fertilizers on bacteria catalyzing P turnover in agricultural soils”. *Agriculture, Ecosystems & Environment*, 107419. https://doi.org/10.1016/j.agee.2021.107419
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  (P5, co-author, accepted)

Publication 1

Biocrusts: Overlooked hotspots of managed soils in mesic environments

Corinna Gall, Juliette Ohan, Thomas Scholten, Michael Schloter, Stefanie Schulz, Steffen Seitz, **Julia Katharina Kurth**

Short description:

Biocrusts are well-known hotspots from drylands, where they display the dominant vegetation form and strongly influence biogeochemical cycles. This viewpoint demonstrates that biocrusts are also widespread in mesic areas, particularly where the impact of humans and soil surface disturbances are high. Still, their influence needs to be better understood. Little is known about the duration of their existence in highly disturbed areas. Therefore, it is essential that future research uses interdisciplinary approaches to investigate the mechanisms of their formation and persistence, their impact on ecosystems, and possible opportunities and risks of their spread, especially with regard to increasing droughts in the context of climate change.

Contributions:

- Contributed to the idea and concept of the publication
- Contributed to literature research
- Contributed to manuscript writing

Publication 2

Correlation of the abundance of bacteria catalyzing phosphorus and nitrogen turnover in biological soil crusts of temperate forests of Germany

Julia Katharina Kurth, Martin Albrecht, Ulf Karsten, Karin Glaser, Michael Schloter, Stefanie Schulz

Short description:

From drylands, biocrusts are known to form hotspots of nutrients and microorganisms, which strongly influence nutrient pools of soils and *vice versa*, microbial communities are shaped by nutrient pools. To investigate the influence of bacterial communities in biocrusts on biogeochemical cycles in temperate regions, biocrusts and bare soils from temperate beech forests of Germany with different parent soil materials were sampled. The data indicated an increased abundance of bacteria involved in phosphorus turnover. The abundance of bacteria involved in nitrogen turnover was only increased in biocrusts from soils with low nutrient contents. Phosphorus and nitrogen turnover were closely correlated on sites with increased labile pools of both nutrients. However, the strategies for nutrient acquisition in biocrusts are strongly driven by the soil properties.

Contributions:

- Contributed to experimental planning and design
- Performed sampling, sample preparation, and qPCR measurements
- Performed statistical analysis, data visualization, and interpretation
- Wrote the manuscript

Publication 3

Biological soil crusts on agricultural soils of mesic regions promote microbial cross-kingdom co-occurrences and nutrient retention

Julia Katharina Kurth, Martin Albrecht, Karin Glaser, Ulf Karsten, Gisle Vestergaard, Martin Armbruster, Susanne Kublik, Christoph A. O. Schmid, Michael Schloter, Stefanie Schulz

Short description:

Biocrust are well known from (semi-) arid areas where they develop over long periods and experience little disturbances. Very little is known about human-impacted sites with frequent disturbances. The question was raised if biocrusts from mesic regions of managed sites develop similarly to their better-known counterparts from drylands. Furthermore, the impact of fertilization and tillage regimes on microbial community composition and structure were analyzed from biocrust and bare soil samples taken from a long-term agricultural experimental site with particular low soil quality. Interestingly, increased amounts of nutrients, phototrophic biomass, and microorganisms were discovered in biocrusts compared to bare soils, no matter which fertilizer or tillage regime was applied. Network complexity was reduced by tillage, while it was increased by fertilization with mineral or organic components. Interestingly, Cyanobacteria were not among the network hubs in biocrusts but have been detected as the most connected group in bare soil networks why it is assumed that Cyanobacteria are essential for the initialization of biocrust in managed, mesic environments.

Contributions:

- Performed sample preparation and qPCR measurements
- Prepared amplicon libraries and bioinformatics analysis
- Performed network analysis
- Performed statistical analysis, data visualization, and interpretation
- Wrote the manuscript

1. Introduction

Biological soil crusts, hereafter biocrusts, are found all over the world, account for 12 % of the terrestrial landmass (Rodriguez-Caballero *et al.*, 2018), and have been studied for decades (Belnap, 2003; Büdel *et al.*, 2009; Castillo-Monroy *et al.*, 2010; Eldridge and Greene, 1994; Fischer, Veste, Schaaf, *et al.*, 2010; Jung *et al.*, 2018; Lange *et al.*, 1997; Mayland *et al.*, 1966; Nagy *et al.*, 2005; Rushforth and Brotherson, 1982; Schulz *et al.*, 2016; Williams *et al.*, 2017; Yeager *et al.*, 2004). Biocrusts are an assemblage of soil particles, phototrophic and heterotrophic organisms on the uppermost millimeters of often nutrient-poor, non-aggregated bare soils without higher vegetation and appear as pioneer communities. They are particularly important in dry ecosystems because they are crucial in nutrient cycling, soil stabilization, and water infiltration (Belnap and Lange, 2003; Weber *et al.*, 2022).

The properties and structure of biocrusts result from biological processes associated with the colonization of organisms and not, like physical soil crusts, through processes such as physical soil aggregation or shrinking and swelling caused by drying-wetting cycles (Figure 1). Like in a biofilm, colonizing microorganisms embed themselves in a self-produced matrix of extra-polymeric substances, which enables interactions through the exchange of metabolites among the microorganisms (Flemming *et al.*, 2016; Hall-Stoodley *et al.*, 2004; Vlamakis *et al.*, 2013). Furthermore, the organisms in biocrusts are protected by the EPS from drying out, which is important as they are regularly desiccated, in contrast to aquatic biomats (Weber *et al.*, 2022). Additionally, biocrusts do not colonize on top of the soil surface but incorporate soil particles in their structure.

As they mainly occur in areas where the growth of higher plants is not possible, they are often extremotolerant communities, which can resist high radiation, cope with little water availability, and withstand desiccation (Belnap *et al.*, 2001). On the one hand, some organisms are able to migrate to lower parts of the biocrusts to protect themselves from UV light. On the other hand, dark pigments can function as radiation protectors (Belnap and Lange, 2003). Furthermore, inhabiting organisms developed the ability to resist dry-wetting cycles (Belnap *et al.*, 2001; Hoek *et al.*, 1995).

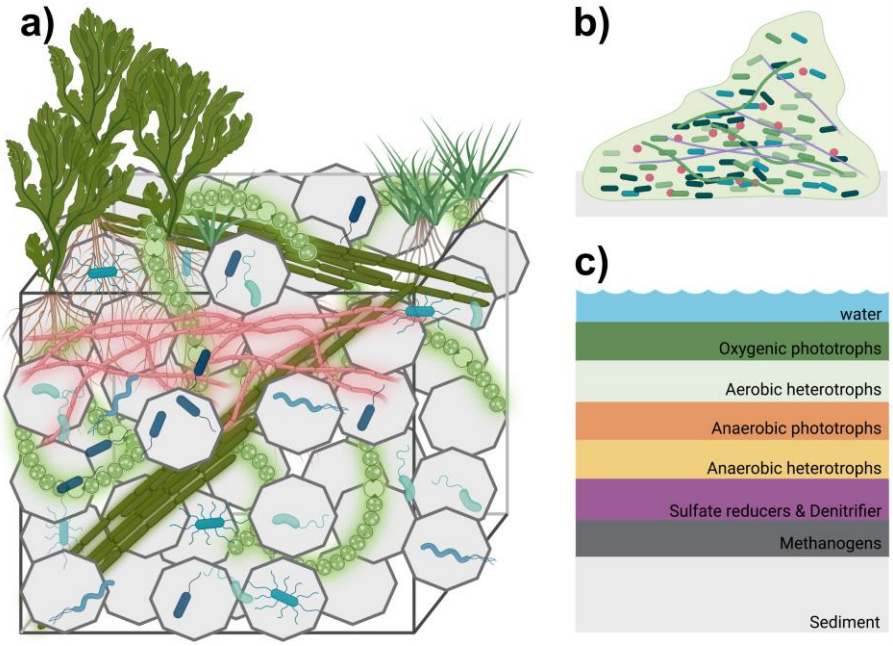

Figure 1 Schematic differentiation between biocrusts, biofilms, and aquatic biomats. a) Figure of a biocrust including phototrophic organism (green) like cyanobacteria, e.g., *Microcoleus* sp., *Nostoc* sp., algae, or mosses, microbial cells (blue) and fungal hyphae (red). Soil cube of about 3 mm size. Adapted from Belnap and Lange (2003) and Weber *et al.* (2022). b) Figure of a biofilm in the mature stage, adapted from Flemming and Wuertz (2019). c) Figure of an aquatic biomat with different layers, among others derived from an oxygen gradient, adapted from Prieto-Barajas *et al.* (2018).

1.1. Structure of biocrusts

1.1.1 Phototrophic community composition

Dominating phototrophic groups of biocrusts are cyanobacteria, algae, mosses, or lichens (Belnap *et al.*, 2001; Eldridge and Greene, 1994; Lange *et al.*, 1997; Mayland *et al.*, 1966; Rushforth and Brotherson, 1982).

Cyanobacteria are among the very first colonizers of bare soils and have been found to dominate phototrophic organisms and members of biocrusts worldwide (Warren *et al.*, 2019). The filaments of cyanobacteria, specifically within the genus of *Microcoleus*, wind throughout the soil particles and aggregates them through the excretion of the sticky sheath (Belnap *et al.*, 2016; Belnap and Lange, 2003; Garcia-Pichel and Wojciechowski, 2009). Also, members of the genus of *Nostoc* are common in biocrusts and known to excrete EPS, which has been found to glue loose soil particles to aggregates (Cania *et al.*, 2019; HuiXia *et al.*, 2007). All cyanobacteria can fix atmospheric carbon with the energy of photosynthesis, and some are even able to contribute to nitrogen fixation, as do *Nostoc*, *Chroococcidiopsis*, or *Scytonema*, for example (Belnap *et al.*, 2016; Berman-Frank *et al.*, 2003; Joshi *et al.*, 2020; Kumar *et al.*, 2010; Sánchez-Baracaldo and Cardona, 2020).

Just like the cyanobacteria, filamentous growing groups of algae, like the eukaryotic algae *Klebsormidium* or *Zygonium*, are found frequently in biocrusts since they also hold soil particles together with their filaments and mucilage (Glaser *et al.*, 2018). Furthermore, many coccoid algae could be detected in the biocrusts (Belnap and Lange, 2003; Schulz *et al.*, 2016). Biocrusts from coastal dunes also comprised diatom species (Schulz *et al.*, 2016). In later stages of succession, when the soil surface is stabilized, mosses and lichens colonize biocrusts (Belnap and Lange, 2003). The dominance of a phototrophic group has been shown to depend on various factors such as geographical position, water availability, pH, soil substrate, or stage of succession (Belnap *et al.*, 2016; Benavent-González *et al.*, 2018; Maier *et al.*, 2018; Xiao and Veste, 2017).

1.1.2 Non-phototropic community composition

With increasing biocrust maturity, nutrient contents increase and alter habitat conditions. The nutrient input attracts heterotrophs (Maier *et al.*, 2018). This leads to higher microbial biomass and diversity along biocrust succession and more complex interaction patterns compared to bare or bulk soil without biocrusts (Chilton *et al.*, 2018; Garcia-Pichel *et al.*, 2003; Maier *et al.*, 2018; Xiao *et al.*, 2022). It was reported that the changes in microbial community composition during succession are traced back to altered soil properties, increasing nutrient contents, and the stabilization of loose material, which created a protected space attracting microorganisms like heterotrophic bacteria or fungi (Cania *et al.*, 2019; Costa *et al.*, 2018; Mazor *et al.*, 1996; Rossi *et al.*, 2018).

Common microbial members of dryland biocrusts are frequently observed in soil samples as well and belong to the archaeal phyla of Cren- and Thaumarchaeota and the bacterial phyla Acidobacteria, Actinobacteria, Armatimonadetes, Bacteroidetes, Chloroflexi, Gemmatimonadetes, Proteobacteria, Planctomycetes, Verrucomicrobia (Maier *et al.*, 2018; Nagy *et al.*, 2005; Pombubpa *et al.*, 2020). Further, Fungi have been detected in biocrusts and include free-living and mycorrhizal groups belonging to Ascomycota, Basidiomycota, Calcarisporiellomycota, Chytridiomycota, Mortierellemycota, Rozellomycota (Abed *et al.*, 2019; Nevins *et al.*, 2021; Pombubpa *et al.*, 2020; Warren *et al.*, 2019). Specifically, copiothrophs often increase in biocrusts as they profit from increased nutrient contents (Couradeau *et al.*, 2019; Glaser, Albrecht, *et al.*, 2022). The increased abundance of organisms further attracts bacterivore groups (Glaser, Albrecht, *et al.*, 2022). Next to microorganisms, biota such as protists (Roshan *et al.*, 2021), nematodes (Ngosong *et al.*, 2020), or microarthropods (Belnap and Lange, 2001) were also found to live in sheltered biocrusts.

1.1.3 Microbial cooperation

Furthermore, it is critical to identify mutual interactions among organisms, as diversity alone does not fully reflect the organization of communities (Shi *et al.*, 2016). Complex microbial communities, like soils, have been explored with network analyses, where correlation patterns reveal potential influences of different groups on each other (Barberán *et al.*, 2012; Berry and Widder, 2014; Faust and Raes, 2016). On the one hand, a positive correlation displays potential interaction, which could be traced back to shared niches (Shi *et al.*, 2016). For example, it has been observed that the degradation of organic matter is carried out together by different microbiological groups in a cascade, where Archaea, Bacteria, and Fungi profit from each other (Velmourougane *et al.*, 2017; de Vries and Wallenstein, 2017). On the other hand, negative correlations display mutual exclusions as they could occur when taxa feed on the same nutrients, but one is more successful and suppresses the other (Kramer *et al.*, 2020). First attempts were made to examine the structure of biocrust microbial communities by network calculations, where Actinobacteria, Proteobacteria, Cyanobacteria, and Ascomycota functioned as hubs among all phyla (Pombubpa *et al.*, 2020). Cyanobacteria were recently described as keystone species in biocrusts and were found to act as network hubs in early-stage biocrusts (Chilton *et al.*, 2018). Furthermore, they form several important inter-kingdom interactions with heterotrophic bacterial phyla such as Actinobacteria, Proteobacteria, and Bacteroidetes (Couradeau *et al.*, 2019) and Fungi like Dothideomycetes, Eurotiomycetes, or Sordariomycetes (Chilton *et al.*, 2018; Pombubpa *et al.*, 2020). In lab experiments, Couradeau *et al.* (2019) were even able to show that heterotrophic groups profit directly from autotrophic nutrient accumulation: Copiotrophs were associated with filaments of the Cyanobacterium *Microcoleus vaginatus*, which is very abundant in biocrusts (Couradeau *et al.*, 2019).

1.2. Functions of biocrusts

Biocrusts have been shown to contribute to several ecologically relevant functions and ecosystem services (Belnap *et al.*, 2016). Together with the filaments of cyanobacteria, algae, or fungal hyphae, the EPS matrix holds soil particles together and, like this, protects soil surfaces from erosion (Belnap *et al.*, 2001; Eldridge and Greene, 1994). These sticky polymers and the physical structure of biocrusts additionally capture and store moisture and reduce the loss of water or nutrients (George *et al.*, 2010). Since biocrusts can colonize particularly well on nutrient-poor substrates, their carbon and nitrogen input from fixing atmospheric CO_2 and N_2 due to biological processes plays a crucial role, and they contribute 7 % of global carbon fixation by terrestrial vegetation and approximately 50 % of nitrogen fixation (Elbert *et al.*, 2012). Although phosphorus cannot be fixed from the atmosphere, it was observed that the proportion of organic phosphorus was increased in biocrusts compared to the underlying bulk

soil (Baumann *et al.*, 2017). In building up biomass, the nutrients are first immobilized and protected from leaching or sorption, but due to cell death or decay, they become available for other organisms (Belnap and Lange, 2003).

1.2.1 Carbon turnover

Biocrusts are initialized and characterized by their phototrophic members. This is particularly relevant as carbon - apart from carbonate ($CaCO_3$), which fulfills an important buffer function in the soil - cannot be gained from pedogenesis by weathering of minerals of the soil (Blume *et al.*, 2010a). During photosynthesis and carbon fixation, through the conversion of solar energy to chemical energy, carbon dioxide (CO_2) and water (H_2O) are transformed into glucose ($C_6H_{12}O_6$) and oxygen (O_2) (Figure 2). Therefore, wetting events instantly activate desiccated biocrusts and start photosynthesis and biomass production (Fernández-Marín *et al.*, 2016). Chlorophyll harboring species absorb solar energy in the photosystem, which catalyzes the conversion of water to oxygen, starting a cascade of electron transfers leading to the formation of glucose-building ATP (Ferreira *et al.*, 2004; Jones and Fyfe, 2001; Jordan *et al.*, 2001). During various metabolic processes, it is incorporated into organic forms such as polysaccharides, fatty acids, chitin, or lignin and contributes to the total carbon stock of soils (Blume *et al.*, 2010a; Six *et al.*, 2002). Most biological systems are limited in their growth due to the lack of carbon availability (Hodge *et al.*, 2000). Its presence primes the growth of organisms and can even lead to the formation of local microbial hotspots (Kuzyakov and Blagodatskaya, 2015).

1.2.2 Nitrogen turnover

Like Carbon, nitrogen is very limited in parent rock material because the main natural input paths to the soil are - like for carbon - aerial deposition, fixation by organisms, or transformation of organic substances. Nitrate (NO_3^-), ammonium (NH_4^+), and organic substances like proteins or nucleotides are relevant nitrogen species. The first two display the primary bioavailable forms for organisms. Nitrate can easily be leached from the soil because it has a high solubility in water and low binding to the soil (Blume *et al.*, 2010b; Marschner, 2011).

In biocrusts, diazotrophs perform the transformation of atmospheric nitrogen (N_2) to ammonium (NH_4^+) as free-living, plant-associated, or symbiotic bacteria or archaea (Figure 2). The fixation of nitrogen is a high energy-demanding process as it consumes at least 16 ATP molecules. Therefore, it is known to occur mainly in nitrogen-limited systems, like most dryland biocrusts. (Zehr *et al.*, 2003). The nitrogenase enzyme complex (EC 1.18.6.1) catalyzes the

reduction of N_2 by using component I, encoded by *nifD* and *nifK* genes, and component II performing the ATP hydrolysis, encoded by *nifH* gene (Burgess and Lowe, 1996; Newton, 2007). Since this reaction requires anaerobic or microaerophilic conditions, various mechanisms protect nitrogenase from oxygen in diazotrophs. For example, cyanobacteria developed heterocysts for nitrogen fixation where no photosynthesis occurs, as observed in *Anabaena*, *Nostoc*, *Scytonema,* and others (Fay, 1992; Harper and Marble, 1988). Inoculation experiments revealed that cyanobacteria in biocrusts can not just provide nitrogen but also retain more nitrogen than non-inoculated ones, increasing their potential use in agriculture to help mitigate the loss of nitrogen (Peng and Bruns, 2019).

With increasing ecosystem maturity, the nitrogen stock and microbial biomass increase, resulting in more organic nutrients, including proteins and chitin (Cohen-Kupiec and Chet, 1998; Schulten and Schnitzer, 1997). Bacteria further fulfill their nutritional requirements by degrading organic material. Extracellular proteases and chitinases are responsible for their breakdown (Geisseler *et al.*, 2010). Proteases are subdivided into neutral, alkaline, and acidic proteases according to their pH optimum. Alkaline (Apr) and neutral (Npr) metalloproteases (EC 3.4.24) are common in bacteria and mainly cause protein breakdown in beech forests and arable soil (Bach and Munch, 2000; Kalisz, 1988). Chitinases (EC 3.2.1.14) play a crucial role in the natural recycling of chitin in nature and serve as a nutrient source for bacterial growth. They are classified in families 18 and 19, where most of the latter occurs in plants. Family 18 is subdivided into three groups: A, B, and C, whereas group A (ChiA) is most commonly observed across different environments (Xiao *et al.*, 2005).

1.2.3 Phosphorus turnover

Unlike carbon and nitrogen, organisms cannot fix phosphorus from the atmosphere, and though the primary source for organisms is the direct uptake of orthophosphate from the soil solution, the transformation of organic substances or the solubilization of adsorbed P (Richardson and Simpson, 2011). Therefore, it is interesting that the proportion of organic phosphorus was increased in biocrusts compared to the underlying bulk soil, even if the total phosphorus contents were not. Hence it was hypothesized that members of biocrusts are responsible for the transformation between pools (Baumann *et al.*, 2017).

In soils, phosphorus can be found in a soluble form in the soil solution, sorbed to minerals, occluded in these, as defined phosphorus minerals, or bound in organic substances and soil organisms (Blume *et al.*, 2010b; Walker and Syers, 1976). Phosphorus sorbing minerals in soils are iron and aluminum oxides or clay minerals and, depending on pH, calcium (Ca) and magnesium (Mg) carbonates (Holford, 1997). It is observed that the amount of soil phosphorus

occluded in minerals increases with time and becomes only hardly accessible (Holford, 1997; Vitousek *et al.*, 2010; Walker and Syers, 1976). However, the bulk soil phosphorus concentration greatly influences the proportional contribution to phosphorus pools (Aciego *et al.*, 2017). Orthophosphate is the primary form of phosphorus that bacteria can take up (Santos-Beneit, 2015). However, bioavailable orthophosphate concentrations can be low, even if the soil contains large amounts of total phosphorus (Rodríguez *et al.*, 2007). Microorganisms are well-accepted to influence phosphorus turnover and its availability in soils (Richardson and Simpson, 2011).

Microorganisms developed various paths to access the diverse phosphorus pools. Metagenome analysis of bacterial phosphorus turnover in mesic forest ecosystems recently revealed the most important processes for solubilization, mineralization, or phosphorus uptake (Figure 2) (Bergkemper, Schöler, *et al.*, 2016). Next to other possible mechanisms, the quinoprotein glucose dehydrogenase (Gcd, EC 1.1.5.2) solubilizes mineral phosphorus. Bacteria use Gcd to dissolve and assimilate mineral phosphorus via non-phosphorylating glucose oxidation in the periplasmic space (Goldstein, 1995). However, this natural source is endless to the point where all phosphorus is depleted from primary minerals (Holford, 1997; Vitousek *et al.*, 2010; Walker and Syers, 1976). Therefore, recycling organic matter becomes more important as the pool of organic substances increases with increasing ecosystem development. Dominating processes were the recycling of phosphomonoesters or -diesters by acid (Nonspecific acid phosphohydrolases, PhoN, EC 3.1.3.2) or alkaline (PhoD, EC 3.1.3.1) phosphatases, of phosphonates by phosphonoacetaldehyde hydrolase (PhnX, EC 3.11.1.1), and of myo-Inositol-1,2,3,4,5,6-hexakisphosphates (IP_6) by 4-phytase (AppA, EC 3.1.3.2, 3.1.3.26). In addition to the transformation processes mentioned above, bacteria developed specific phosphorus uptake systems to compete with other biota, where the dominating ones are the unspecific phosphate inorganic transporter (PitA, K03306) or the starvation-induced phosphate ABC transporter (PstS, K02040) (Hsieh and Wanner, 2010; Wanner, 1993). The latter is additionally part of the regulatory system of the Pho regulon and is its most conserved member (Santos-Beneit, 2015).

1.2.4 Stoichiometric constraints of nutrient turnover

Indeed, microorganisms always seek a stable ratio of nutrients in their biomass, so the uptake of various nutrients underlies stoichiometric constraints (Cleveland and Liptzin, 2007; Finzi *et al.*, 2011). The Pho regulon is not just responsible for regulating phosphorus uptake. It also controls nitrogen transcription regulators, thus, directly interfering with nitrogen pathways (Santos-Beneit, 2015). Microorganisms need the respective other nutrients to build enzymes

acquiring the others, e.g., carbon, to produce acids for P solubilization (Spohn, 2016; Widdig *et al.*, 2019). Thereby, increased net mineralization of nitrogen and phosphorus were strongly correlated in organic horizons from temperate forests (Heuck and Spohn, 2016), and microbial phosphorus and nitrogen have been positively correlated (Sorkau *et al.*, 2018). In line with these findings, metagenomics of arable and forest soils exhibited that bacteria able to fix nitrogen were positively correlated to bioavailable phosphorus (Bergkemper, Schöler, *et al.*, 2016; Grafe *et al.*, 2018).

In addition to bacteria, microbial members of biocrusts also include archaea and fungi. Both influence nutrient pools in the soil as well (Qin *et al.*, 2020; Stempfhuber *et al.*, 2016; Stribley *et al.*, 1980), but their specific impact on carbon, nitrogen, or phosphorus pools was not examined in the context of this thesis. However, since they are essential members of the microbial community of biocrusts, they were included in co-occurrence patterns to analyze the microbial community structure of biocrusts.

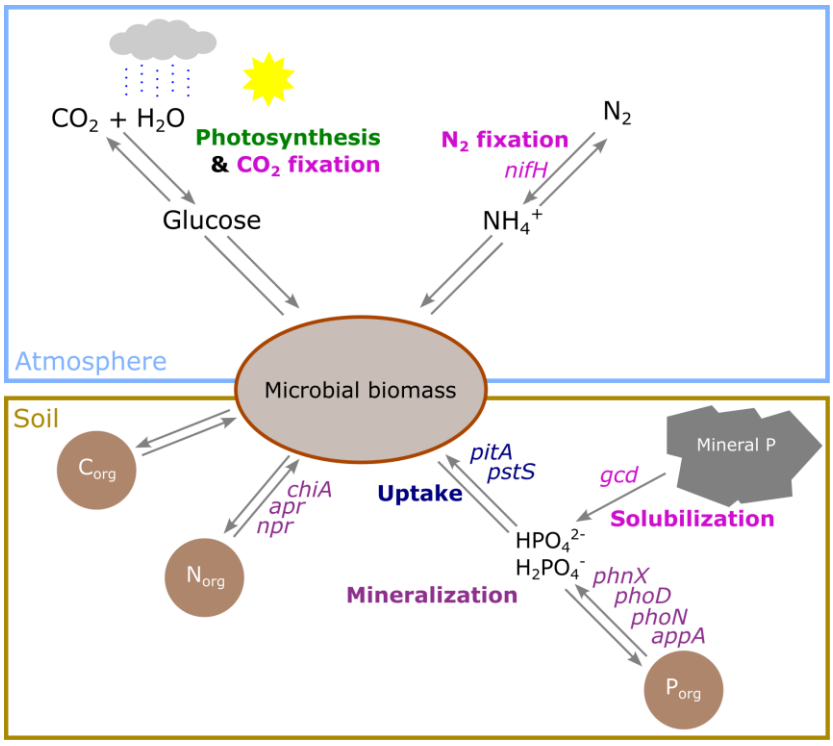

Figure 2 Simplified overview of microbial processes to transform carbon, nitrogen, and phosphorus pools by input (pink), mineralization (purple), and uptake (blue) processes in biocrusts. Other processes shaping nutrient pools in soils are excluded from this figure as they are not part of this thesis. Not included are processes that cause a loss of nutrients like respiration, denitrification or nitrate leaching, or the organic turnover of carbon.

1.3. Factors driving biocrust communities

1.3.1 Geographical distribution

Biocrusts are distributed over the whole globe and, in drylands, are formed everywhere where bare soil exists, sunlight reaches the soil, and vascular plant growth is sparse or widely spaced (Belnap, 2006). In these often extreme environments, biocrusts are the final successional stage because vascular plants cannot thrive here (Belnap *et al.*, 2001). Thus, biocrusts are widespread in drylands of arid or semiarid regions like deserts of the USA in Colorado (Garcia-Pichel *et al.*, 2003), Arizona (Nagy *et al.*, 2005), Utah (cold desert) (Ayuso *et al.*, 2017), California (Mogul *et al.*, 2017), in China (Liu *et al.*, 2017), and Australia (Eldridge and Greene, 1994). Drylands encompass more than 40 % of the Earth's total terrestrial land area (Belnap, 2006), and large parts of them are covered by biocrusts (Bowker *et al.*, 2002).

Furthermore, they have been detected in polar (Rippin *et al.*, 2018; Williams *et al.*, 2017) and alpine regions (Brankatschk *et al.*, 2011; Mikhailyuk *et al.*, 2015) because of reduced higher vegetation due to low temperatures and/or high elevation levels. In addition, biocrusts have been identified in nutrient-poor sites in the Mediterranean and temperate regions like coastal or inland dunes, former mining or reclamation sites, or even forests (Büdel, 2001; Cania *et al.*, 2019; Corbin and Thiet, 2020; Gypser *et al.*, 2016; Pushkareva *et al.*, 2021; Schulz *et al.*, 2016).

In mesic regions, where higher plants are temporarily removed due to natural or anthropogenic processes, biocrusts can also find a temporal niche to develop (Warren *et al.*, 2019). Few studies have investigated biocrusts in managed areas where anthropogenic influences dominate, like agricultural systems or forests (Baumann *et al.*, 2017; Glaser *et al.*, 2018, 2021; Nevins *et al.*, 2020, 2021).

1.3.1 Disturbance

Biocrusts have been detected as very sensitive to disturbances (Weber *et al.*, 2016). The most commonly described disturbances are nutrient deposition, fire, or various types of surface distraction like grazing or trampling of animals or humans (Cole, 1990; Concostrina-Zubiri *et al.*, 2013).

The nutrient deposition accelerated the succession of microbial communities in the recently deglaciated soils of Peru and changed their community composition (Knelman *et al.*, 2014). In Mediterranean drylands, on the one hand, increased nitrogen deposition reduced bacterial and, even more interesting, cyanobacterial abundance. On the other hand, the abundance of green algae was increased along the nitrogen gradient, leading to a shift in the phototrophic

community (Ochoa-Hueso *et al.*, 2016). Lab cultivation experiments revealed that cyanobacterial nitrogen fixation was decreased (4 to 5-fold) in the presence of nitrate from the growth medium. However, it was not reduced to zero and continued on a lower level (Peng and Bruns, 2019).

Surface disturbance of biocrusts is often investigated together with their ability to recover. Due to their limitation in water and nutrients, biocrusts in (semi-)arid and/or nutrient-poor areas have very long development times. Therefore, their recovery time can take several decades, depending on the timing, type, and severity of the disturbance (Weber *et al.*, 2016). Long-term disturbances cause much higher recovery times than low-intensity events (Belnap *et al.*, 1993; Briggs and Morgan, 2012). Like this, intensive trampling of animals reduces biocrust cover immensely and can even negatively affect their ability to recover after grazing (Concostrina-Zubiri *et al.*, 2013; Williams *et al.*, 2008). Even more severe is the removal of biocrusts by agricultural practices such as plowing, where the entire surface is turned upside down, thereby leading to the disappearance of biocrust cover (Daryanto *et al.*, 2013). The complete removal of biocrusts was detected to have more severe effects than scalping, where the soil surface is not removed (Weber *et al.*, 2016).

In drylands with low nutrients and water availability, the recovery rates can take several years to decades (Weber *et al.*, 2016). Since the recovery rates are very long, several attempts have been made to grow crusts and use them for regeneration (Antoninka *et al.*, 2016; Briggs and Morgan, 2012; Bu *et al.*, 2018; Giraldo-Silva *et al.*, 2019; Tucker *et al.*, 2020). Interestingly, the recovery time can be reduced to a few months when the disturbance is applied before the rainy season (Dojani *et al.*, 2011). That means that recovery is described to be increased with higher precipitation and/or lower disturbance frequency or intensity (Weber *et al.*, 2016).

1.4. Biocrust succession

Biocrusts have been found to undergo a constant succession of the dominating phototrophic group, which changes over time. The succession depends on factors such as climate, water availability, disturbance frequency, or soil texture, where a finer soil texture favors more stable crusts (Weber *et al.*, 2016). Successional stages of the phototrophic community classify biocrusts (Belnap *et al.*, 2001; Maier *et al.*, 2018). Autotrophic organisms like cyanobacteria often dominate the initial stage, followed by green algae-, lichen- and moss-dominated biocrusts. As biocrusts grow, the chlorophyll content – a proxy for active photosynthetic biomass – increases and shows the highest values in moss-dominated crusts (late successional stage) (Maier *et al.*, 2018; Román *et al.*, 2019). The changing communities alter the soil surface by changing its roughness due to the aggregation of soil particles. This also

results in a varying thickness from 0 to 2 mm in light cyanobacterial or algal crusts and up to 6 to 150 mm for pinnacled crusts (Weber *et al.*, 2016). The altered surface roughness consequently influences water-holding capacity. It has been observed that, depending on their successional stage, biocrusts can protect water from evaporation and positively influence water infiltration but can also promote runoffs due to a sealed surface (Belnap *et al.*, 2001; Bowker *et al.*, 2008; Faist *et al.*, 2017; Fischer, Veste, Wiehe, *et al.*, 2010). When disturbance events occur, this succession pattern can be altered or set back to initial developmental stages, which were dominated by cyanobacteria (Belnap *et al.*, 2016; Bu *et al.*, 2013; Ferrenberg *et al.*, 2015; Kuske *et al.*, 2012; Lange *et al.*, 1997; Maier *et al.*, 2018; Steven *et al.*, 2015). Recent literature further revealed different succession patterns depending on the region. Biocrusts in temperate regions are frequently initiated by algae instead of cyanobacteria (Glaser *et al.*, 2018), which might result from precipitation being positively correlated to the growth of algae (Hu and Liu, 2003). Even if the dominant phototrophic group defines the different successional stages, this does not lead to a displacement of other phototrophic groups but to a shift in relative abundances (Weber *et al.*, 2016). Like this, a lasting increase of cyanobacterial abundance during biocrust development in temperate regions was observed, with algae or mosses dominating the phototrophic biomass of later stages (Cania *et al.*, 2019).

1.5. Aims and Hypotheses of the Ph.D. thesis

Biocrusts have been investigated for decades in (semi-) arid areas worldwide and are known to influence ecosystem functioning and dynamics. Although they also occur in mesic environments, their importance and functions are poorly investigated.

As part of the project “Crustfunction II”, funded by the German Research Foundation, this thesis aimed to characterize microbial communities from biocrusts of managed, mesic forest sites and agricultural fields and detect similarities and differences to the better-known biocrusts from drylands. To understand the influence of microbial members of biocrusts from managed, mesic sites on biogeochemical cycles, in-depth microbial and molecular analysis, including microbial correlation networks and quantification of functional groups, and determination of nutrient pools was performed.

In drylands, biocrusts display the final successional stage and were found to be limited in development and growth by disturbance events. Due to the limited water supply, regeneration is very slow and takes up to decades. Contrary to this, mesic environments are characterized by a good moisture supply and are dominated by higher plants. In these environments, their limitations are expected to be very different. Before biocrusts can grow, plant or litter cover must be removed to create bare soil. It is hypothesized that contrary to drylands, biocrusts of mesic environments need disturbance events to have a chance to develop and then develop within one growing season due to the temperate growth condition (**H1**).

Biocrusts of drylands are hotspots of nutrients and diversity because the stabilization of loose soil and the phototrophic and diazotrophic activity attracts heterotrophic organisms. Despite the decent nutrient supply in mesic, managed areas, biocrusts are expected to develop into hotspots of nutrient turnover, microbial abundance, and functional gene abundances because the availability of carbon primes microbial activity and the filamentous growth and EPS excretion further stabilizes the soil and creates a protected micro-habitat (**H2**).

In addition, it is well known that microorganisms take up nutrients in parallel to keep stable stoichiometric ratios in their cells. Therefore, the turnover of individual nutrients is expected to increase, and the acquisition processes are closely interlinked. Thus, the mineralization of organic matter is equally subject to replenishing nitrogen and phosphorus. The cooperating habitat of biocrusts further facilitates positive cross-kingdom interactions. Hence, biocrusts support the interaction between taxonomic and functional groups (**H3**).

2. Material and Methods

This thesis focuses on biocrust communities and their differentiation from bare soil in forests and agricultural fields in mesic environments. Field samples were taken from long-term research projects in Germany. The interaction of bacterial-driven nitrogen and phosphorus turnover determined by quantification of functional genes with quantitative polymerase chain reaction (qPCR) and its link to the nutrient pools was investigated in beech forests within a gradient of nutrient stocks (**P2**). The effect of agricultural management on the archaeal, bacterial, and fungal community composition as well as on the interaction of microorganisms, was analyzed on a sugar beet field by qPCR and sequencing of 16S rRNA and ITS amplicons, as well as disentangling their community structure by network analysis and the quantification of phototrophic biomass by measuring chlorophyll *a* content (**P3**). Microbial analysis was accompanied by determining abiotic soil properties such as soluble carbon (**P3**), nitrogen (**P2**, **P3**) and phosphorus (**P2**), as well as by determining the pH (**P2**, **P3**).

2.1. Experiments

2.1.1 Experiment 1 – Biocrusts from forest soils

Biocrust samples for this study were taken within the frame of the Biodiversity Exploratories (www.biodiversity-exploratories.de; (Fischer, Bossdorf, *et al.*, 2010; Fischer, Kalko, *et al.*, 2010)). This project was established from 2006 to 2009 in three regions across Germany: the Biosphere Reserve Schwäbische Alb (Alb) in the Southwest, the National Park Hainich-Dün (Hainich) in central Germany, and the Biosphere Reserve Schorfheide-Chorin (Schorfheide) in the Northeast (Figure 3). The sites display a north-to-south gradient in soil texture, pH, nutrient status, and annual precipitation (Alt *et al.*, 2011; Grüneberg *et al.*, 2010). The exploratories allow functional biodiversity research on a large scale on actual farmed areas, where each study region consists of 50 grassland and 50 forest plots with varying land use intensities. In grasslands, this is defined by fertilizing, mowing, and grazing frequencies (Blüthgen *et al.*, 2012). Forest plots differ in silvicultural management intensities defined by “tree species, stand age and aboveground, living and dead wooden biomass”

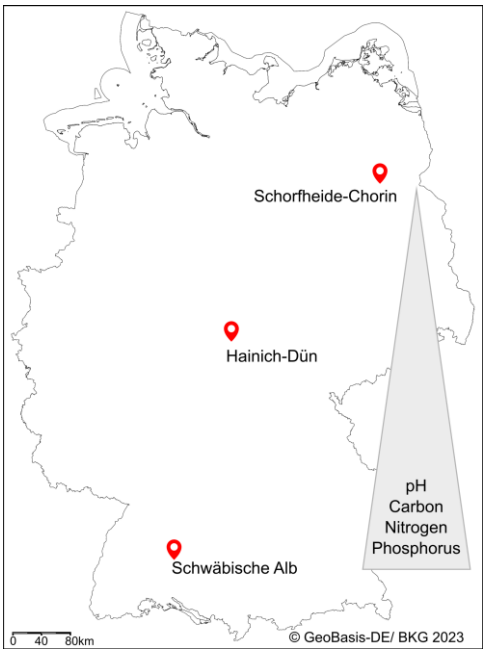

Figure 3 Location of the Biodiversity Exploratories in Germany: Schorfheide-Chorin is in Brandenburg, Hainich-Dün in Thuringia, and Schwäbische-Alb in Baden Wurttemberg.

(Schall and Ammer, 2013). In the reserve of Schorfheide and Hainich, also unmanaged beech forests exist.

Nutrient and water supply in grasslands of mesic regions nutrient and water supply is rather good why weeds quickly emerge, and biocrusts are rare due to the missing bare soil. Therefore, this thesis focused on the forest plots within the Biodiversity Exploratories. All 150 forest plots in Alb, Hainich, and Schorfheide have been visited. Biocrusts were discovered on beech (*Fagus sylvatica* L.), pine (*Pinus sylvestris* L), and oak (*Quercus robur* L. and *Quercus petraea* L) sites, which are the most dominant tree species in forests of the Biodiversity Exploratories. When light reaches the soil, forest soil surfaces are often covered by litter, grasses, and bushes. Bare soil and biocrusts were detected where the disturbance was caused naturally by, e.g., animals digging holes or fallen trees, or anthropogenically by forest management, which causes skid trails and prevents the growth of higher plants.

To sample at the peak of biocrusts occurrence, sampling took place in April/May 2018 before plants came into the bud and biocrusts could develop after winter. Wherever biocrusts were discovered, up to six replicates per plot were sampled. A decent number of biological replicates are needed for sufficient statistical power for the analysis. Therefore, it was necessary to subset the complete sample set after the campaign and discard samples without a decent number of replicates. Consequently, samples taken on skid trails from even-aged, managed beech plots remained. This resulted in a total amount of 27 spots together in all three exploratories (11 in Schwäbische Alb, 9 in Hainich, and 7 in Schorfheide Chorin).

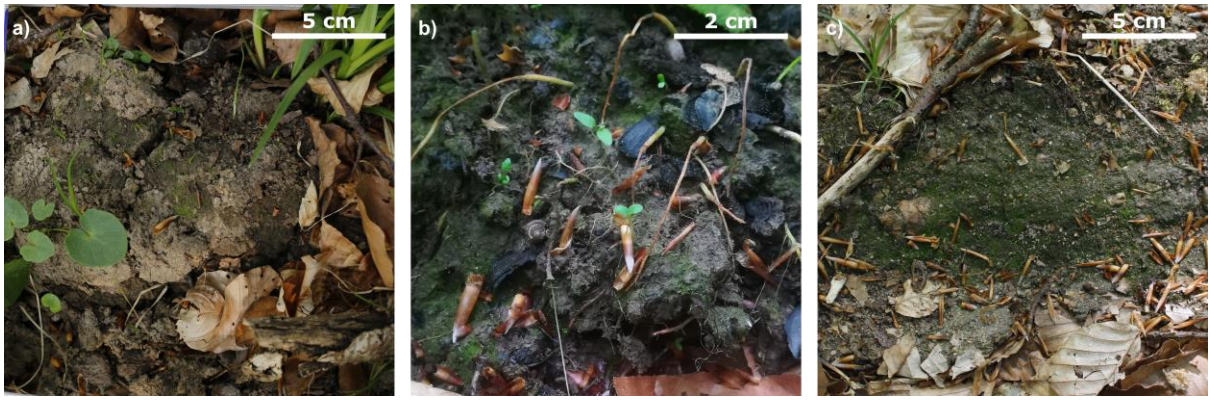

Figure 4 Biocrust on forest soil between beech trees. Pictures show different Biocrusts in A) Schwäbische Alb, B) Hainich, and C) Schorfheide-Chorin.

According to Weber *et al.* (2022), biocrusts, defined as green-covered soil (Figure 4), and biocrust-free bare soil, which acted as a control, were taken with sterile Petri dishes of 10 cm diameter. Petri dishes were further used to transport samples at 4°C to the lab, where the top 5 mm were separated with a razor blade and homogenized prior to storage at -20°C for nutrient

analysis and at -80°C for microbial analysis. Together with the bare soil samples, a total sum of 54 samples (27 pairwise couples of bare soil and biocrust) were chosen for this analysis.

2.1.2 Experiment 2 – Biocrusts from agricultural Soils

Samples were taken within the “International Organic Nitrogen Fertilization Experiment” from the long-term research station Landwirtschaftliche Untersuchungs- und Forschungsanstalt, Rinkenbergerhof, Speyer, Rhineland-Palatinate in Germany (49°19'06.8"N, 8°25'25.8"E, Figure 5) (Armbruster *et al.*, 2012; Bischoff and Emmerling, 1997). In 1983, the experiment was established with a three-year crop rotation of sugar beet, winter wheat, and winter barley to investigate the effect of mineral and organic fertilizer. Since 2004, the impact of tillage has been investigated, as well. The soil type is Cambisol, with a texture of 48.3 % silt, 42.8 % sand, and 8.9 % of clay. The pH is, on average, 6 to 6.5. Germany's arable assessment (“Ackerzahl”) rates this site at 25 to 35. The German system evaluates farmland according to soil quality, site characteristics, and climatic conditions, where the best value to achieve is 100 and represents very fertile sites. Values below 30 represent sites prone to erosion, low fertility, and unfavorable texture. The usable field capacity of approx. 10% and the low groundwater level of 3 m result in an unfavorable water supply for crops. Crops were irrigated if necessary to avoid drought-related crop damage (mean value over the years for winter barley: 30 mm, winter wheat: 65 mm, sugar beet: 168 mm). Furthermore, all plots received fertilization with essential nutrients of 28 kg of magnesium, 31 kg of phosphorus, and 121 kg of potassium ha^{-1} yr^{-1}. (Armbruster *et al.*, 2012; Bischoff and Emmerling, 1997; Schmid *et al.*, 2018).

Figure 5 Location of the Rinckenberger Hof in Germany.

The influence of organic fertilizer is tested with the application of horse manure of 30 t fresh weight ha^{-1} (animal origin), with crop residues left on the field after harvest (plant origin) or where plant residues are removed (control). The effect of mineral fertilizer as Calcium ammonium nitrate is investigated in five levels varying from 0 to 60, 120, 180, and an excessive amount of 240 kg N ha^{-1} yr^{-1}. Frequency and amount per application differed slightly between crops. For sugar beets this was 0, 40 + 20, 80 + 40, 120 + 60 and 160 + 80 kg N ha^{-1} yr^{-1}. The intensity of tillage is run in three variations. Minimal tillage is run with a rotary

harrow at a machining depth of 5 cm, which only serves to allow seeding with a conventional machine. Reduced tillage is applied with a cultivator without soil turning at a depth of 10 cm. Conventional tillage has a plowing depth of 30 cm. The experiment is run in a full-factorial design on plots of 6 m * 7 m (Armbruster *et al.*, 2012).

In the frame of this study, biocrusts from two levels of mineral fertilizer (120 and 240 kg N ha^{-1} yr^{-1}), two types of organic fertilizer (no organic fertilizer (-org) and crop residues (+org)), and two types of tillage (reduced (rT) and conventional tillage (cT)) were sampled. No biocrusts could be found on plots without mineral fertilizer or with minimal tillage due to the heavy growth of weeds. Because of the full-factorial design, this resulted in eight different treatments: cT 120 -org, cT 120 +org, cT 240 -org, cT 240 +org, rT 120 -org, rT 120 +org, rT 240 -org, rT 240 +org. The sampling of biocrusts was performed on 5th October 2016 before the harvesting of sugar beets took place to allow the development and persistence during the growing season and to avoid disturbances by harvesting machines (Figure 6). According to Weber *et al.* (2022), all biocrusts, defined as green-covered soil, from one plot were sampled to a depth of 3 mm with a spatula. Biocrust-free areas were sampled as a control at the same sampling depth and referred to as bare soil. Eight treatments sampled in three replicates for two different compartments resulted in 48 samples. The homogenized composite samples of each plot, respectively of biocrust and bare soil separately, were sieved to a particle size of 2 mm. Samples used for chemical analysis were stored at 4 °C; samples for microbial analysis were immediately frozen on dry ice and later stored at -80 °C.

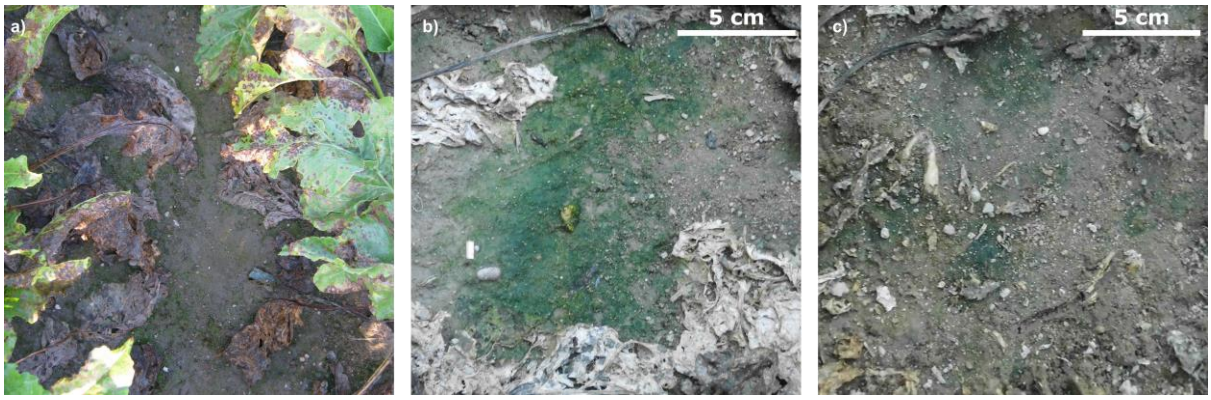

Figure 6 Biocrusts on the Agricultural research farm of Lufa Speyer on IOSDV, taken on October 5th, 2016. a) Biocrust between sugar beets, Biocrust on fields with treatment reduced tillage, 240 N kg ha^{-1} yr^{-1}, and b) without and c) with organic fertilizer (crop residues).

2.2. Analyses of soil parameters

The analysis of soil parameters for experiment 1 (biocrusts from forest soils) was performed by cooperation partners from the University of Rostock.

According to DIN10390 (International Organization for Standardization, 2005), the analysis of pH was performed in an extract of 2 g of fresh soil in 0.01 M $CaCl_2$ solution and measured for experiment 1 with an electrode InLab® Expert Pro and S47 - SevenMulti™ (Mettler Toledo, Columbus, Ohio, United States) and or for experiment 2 with an electrode WTW™ SenTix 61 (Thermo Fisher Scientific, Hampton, New Hampshire, United States) and pH meter (inoLab pH 720 Level 1, Wissenschaftlich-Technische Werkstätten, Weilheim, DE).

For samples of experiment 1, the extraction of nitrate, ammonium, and total dissolved organic nitrogen (DON) was performed on 0.5 g of samples in 20 ml of 0.01 M $CaCl_2$ (Berthold *et al.*, 2015; Brankatschk *et al.*, 2011). A constant flow analyzer (Flowsys, Alliance Instruments, Austria) measured nitrate and ammonium concentration in filtered extracts. The filtrate was further digested with peroxydisulfate for 24 h at 90 °C, neutralized, and then nitrate values measured as above gave total dissolved nitrogen values. DON was calculated as total dissolved nitrogen minus inorganic nitrogen fractions. To determine N_{total}, the sample material was dried (60 °C, 24-48 h), and 30 mg were measured by a CNS-Analyzer (vario EL, Elementar, Germany). To remove carbonates, samples of Alb with an alkaline pH were treated with 10 % HCl and again dried before analysis.

For samples of experiment 2, nitrate, ammonium, DON, and dissolved organic carbon (DOC*)* contents were determined in a 12 ml extract of 0.01m $CaCl_2$ with 3 g of fresh sample material (Brankatschk *et al.*, 2011*).* The filtered extracts (Whatman™ 595 1/2 filters (Sigma-Aldrich, St. Louis, MO, USA)) were analyzed by a segmented flow analyzer (Skalar SANPlus 5100 with autosampler 1050, Skalar analytic, DE, EU) to give nitrate and ammonium and by DIMATOC2000 (DIMATEC Analysentechnik, Essen, DE) to give DOC and DON. Dissolved inorganic carbon (DIC) was calculated as the difference of DOC to the filtrate, where one drop of 32 % HCl was added prior to analysis.

The analysis of inorganic phosphate from samples of experiment 1 was performed on dried (60 °C, 24-48 h) sample material by the molybdenum blue photometric method (Hansen and Koroleff, 1999). Using 5 cm optical glass cuvettes, the absorbance was distinguished at 885 nm by a photometer (UV1200, Shimadzu, Japan). H_2O- (P_{H2O}) and $NaHCO_3$-soluble phosphate (P_{NaHCO3}) were sequentially extracted from 1.5 g of dried material according to the Hedley fractionation (Hedley *et al.*, 1982). Total P (P_{total}) was determined in 100 mg of dried material (60°C, 24-48 h) after digestion with acid peroxydisulfate solution for 24 h at 90°C (Berthold *et al.*, 2015). Neutralization was performed before measurement.

The chlorophyll *a* content as a proxy for phototrophic biomass of samples from experiment 1 was analyzed in an extract of 0.7 g frozen soil of 3 ml 96% aqueous ethanol (v/v) after incubation for 0.5 h at 78 °C and centrifugation at 5°C at 5760 g (Ritchie, 2008). The absorption

was measured at wavelengths of 632 nm, 649 nm, 665 nm, and 696 nm with the spectrophotometer UV-2401PC (Shimadzu, Kyoto, JPN). If chlorophyll was detectable in the supernatant, the extraction steps were repeated. The contents were calculated as the sum of all steps. Chlorophyll contents could not be measured on samples of experiment 2 due to the limited sampling material.

2.3. Analyzing microbial communities in soils

2.3.1 Nucleic acid extraction

DNA of the samples from experiment 1 was extracted based on a phenol-chloroform assay on 0.5 g of frozen material (Griffiths *et al.*, 2000; Töwe *et al.*, 2011). Homogenization and sample lysis were performed in Lysing Matrix Tubes E (MP Biomedicals, USA) with a CTAB solution and Phenol:chloroform:isoamyl alcohol mixture (25:24:1) using the Precellys24 Instrument (Bertin Technologies, France) for 30s at 5500 rpm. Centrifugation at 4 °C at 16 100 x g for 5 min removed soil particles and cell debris. The DNA-containing supernatant was mixed with chloroform:isoamyl alcohol (24:1) to clean from lipids and proteins. PEG solution was added and incubated to enhance DNA precipitation for 2 h. Following the centrifugation for 10 min at 4 °C, the DNA pellet was washed with 70 % ethanol, and the air-dried pellet was eluted in DEPC-treated water.

DNA from samples of experiment 2 was extracted using the NucleoSpin® Soil kit (Macherey-Nagel, Düren, DE) from 0.3 g of frozen soil according to the manufacturer's protocol. After testing the suggested settings, extraction was performed using the lysis buffer SL2 and 150 µl of the enhancer.

Four extraction blanks for each experiment without soil material were processed.

2.3.2 Nucleic acid quantification

The adsorption ratios at 260 nm/280 nm and 260 nm/230 nm were measured with NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific) and used to evaluate the purity of DNA extracts. DNA yields were quantified with a Quant-IT^{TM} Picogreen® dsDNA Assay Kit (Thermo Fisher Scientific). Yields of extraction blanks were below the detection limit, and measurable contamination could be excluded.

2.3.3 Quantification of different prokaryotic target groups by qPCR

The quantification of bacteria carrying specific genes of interest was performed using SYBR Green® based assays (Applied Biosystems, Foster City, CA, USA) on a 7300 real-time qPCR machine (Applied Biosystems, Germany). Each reaction was set to 25 µl consisting of 2 µl of template DNA, forward and reverse primers (10 µM) (Metabion, Planegg, Germany), 3% BSA (Sigma-Aldrich, St. Louis, MO, USA), DEPC-treated water and 12.5 µl of SYBR Green® (Thermo Fisher Scientific). Specific primers, targeted regions, reaction conditions, calibration standards, and thermal profiles are summarized in Table 1. Dilution tests were performed prior to analysis to avoid inhibitory effects. Each run included a standard series ($r^2 > 0.98$, 10^6 to 10^2 gene copies μl^{-1}), no template controls, and samples diluted to 1/64 for experiment 1 and to 1/32 for experiment 2. The quality of qPCR was evaluated by analyzing melting curves and checking randomly chosen samples by electrophoresis on a 1.5 % agarose gel. The amplification efficiency was calculated for each gene by $\varepsilon = 10^{(-1/s)} - 1$ with s = slope of standard series and was measured between 73.9 to 90.35 % for all target groups. If values were below the detection limit of 10 copies (according to the manufacturer's protocol), they were set to *NA*.

Table 1 Primer, reaction conditions, and calibration standards for real-time qPCR.

target gene	Reaction conditions						Calibration standard		
	thermal profile [i]	cycles	BSA 3%	F- and R-primer		reference	ε (%) [ii]	r²	source
16S rRNA gene V5-V6 Bacteria	45s/94 °C, 45s/58°C, 45s/72°C	40	0.5	0.5	FP & RP 16S	Bach *et al.*, 2002	83.71 iii) 77.82 iv)	0.999 0,987	*Pseudomonas putida*
16S rRNA gene V2-V5 Archaea	20 s/95 °C, 60 s/55 °C (*), 60 s/72 °C	5	0.5	0.5	Saf	Nicol *et al.*, 2003	83.88	0,999	*Methanobacterium sp.*
	20 s/95 °C, 60 s/50 °C, 60 s/72 °C	40			958r	Bano *et al.*, 2003			
ITS1 & 2 Fungi	30 s/94 °C, 30 s/50 °C, 30 s/72 °C	40			ITS1, ITS4	White *et al.*, 1990	80,19	0,999	*Trichoderma viride*
nifH	45s/95°C, 45s/55°C, 45s/72°C	40	0.5	0.5	nifH-f & -r Rosch	Rösch *et al.*, 2002	83.68	0.999	*Sinorhizobium meliloti 30136*
apr	45s/95°C, 45s/53°C, 45s/72°C	40	0.5	1	apr-f, apr-r	Bach *et al.*, 2001	84.27	0.999	*Pseudomonas aeruginosa 5071*
chiA	30s/95°C, 30s/60°C, 60s/72°C	40	0.5	1	chiA-f,chiA-r	Xiao *et al.*, 2005	90.35	0.998	*Streptomyces griseus*
phoD	20s/95°C, 60s/60°C, 30s/72°C, 60s/ 81°C	40	0	0.8	phoD-F, phoD-R	Bergkemper, Kublik, *et al.*, 2016	89.55	0.998	*Bradyrhizobium japonicum*
phnX					phnX-F, phnX-R		79.78	0.997	*Salmonella enterica DSM 17058 (DSMZ)*
gcd					gcd-F, gcd-R		82.63	0.996	*Salmonella enterica DSM 17058 (DSMZ)*
pitA					pitA-F, pitA-R		82.03	0.999	*Pseudomonas fluorescens*
pstS					pstS-F, pstS-R		73.92	0.999	*Bradyrhizobium japonicum*

[i] Hotstart for all genes at 95 °C for 10 min

[ii] calculated as $\varepsilon = 10^{(-1/\text{slope})} - 1$

[iii] Experiment 1

[iv] Experiment 2

[v] Touchdown -1°C per cycle

2.3.4 Metabarcoding of archaea, bacteria, and fungi

Archaea and Bacteria were amplified together using the primers Arch0519 F (Klindworth *et al.*, 2013) and Pro 805 R (Herlemann *et al.*, 2011). Fungi were amplified with ITS 3 and ITS 4 (Tedersoo *et al.*, 2015). Illumina overhangs were attached to primers. The reaction mix was set to 25 µl consisting of 1 µl of DNA (3 ng μl^{-1}), forward and reverse primer (10 µM) (Metabion), NebNext® High-Fidelity 2X PCR Master Mix (New England Biolabs, Ipswich, MA, USA), 3% BSA (Sigma-Aldrich) and DEPC-treated water. Details about PCR conditions are summarized in Table 2. Amplification was performed on extraction blanks, PCR negative control, and samples in triplicates. Before pooling, all triplicates were put on 1 % agarose gel to control for amplification success. Agencourt AMPure XP magnetic beads (Beckman Coulter Life Sciences, Brea, CA, USA) performed PCR clean-up in a DNA-to-bead ratio 0.8. Fragment Analyzer™ Automated CE System (Agilent Technologies, Santa Clara, CA, USA) gave DNA concentration and fragment size and was used as a quality check. Multiplexing was performed with Nextera® XT Index Kit v2 (Illumina) in an indexing PCR of 25 µl consisting of 10 ng of purified amplicon, 2.5 µl of indexed forward and reverse primer, and 12.5 µl NebNext® High-Fidelity 2X PCR Master Mix (New England Biolabs). PCR conditions were set to 30 s at 98 °C, 8 cycles of 10 s at 98 °C, 30 s at 55 °C and 30 s at 72 °C and 5 min final elongation at 72 °C. Samples were diluted to 4 nM and pooled to 5 µl of each sample for sequencing with MiSeq Reagent kit v3 (2 x 300 bp, 600 cycles) on the MiSeq® instrument (Illumina), PCR product was cleaned up with AMPure Beads as described previously (Beckman Coulter Life Sciences) and the concentration and quality were determined via Fragment analyzer (Agilent Technologies). As a sequencing control, each run is spiked with 20% PhiX. If samples resulted in less than 10 000 reads, they were re-sequenced.

Demultiplexing of sequencing data was performed by Illumina® MiSeq software, which provided fastq files for forward and reverse reads for each sample with eliminated indices. AdapterRemoval (Schubert *et al.*, 2016) removed Illumina overhangs. DADA2 Version 1.8.0 (Callahan *et al.*, 2016) was used for length and quality filtering within R Version 3.6.1 (R Core Team, 2019). Each read was trimmed at the first 10 base pairs and 250 bp and 200 bp for Bacteria/Archaea and at 275 bp and 225 bp for Fungi, respectively, for forward and reverse reads, according to the DADA2 manual and quality plots produced by DADA2. Quality filtering was done with a maximum of five expected errors per sequence and a minimum phred quality score of two. After this inference step, the provided error plots were used to monitor filtering parameters for the number of reads lost and for fitting the sequence run to the error model. Subsequently, dereplication, denoising, and PhiX and chimera removal are accomplished. This was followed by the alignment of paired forward and reverse reads. Afterward, reads from two runs of the same sample were merged. The inferred amplicon sequencing variants (ASVs)

were used to assign taxonomy by the Silva database Version 132 (Quast *et al.*, 2013) for Bacteria/Archaea and by Unite database Version 7.1 (2016-11-20, Kõljalg et al., 2013) for Fungi.

ASVs in the PCR negative controls or blank extracts and ASVs assigned to Chloroplasts and Mitochondria were removed from all samples. Random subsampling was completed to 19 722 bacterial/archaeal and 22 731 fungal reads, reflecting the lowest number of reads, using the R package phyloseq Version 1.30.0 with the function rarefy_even_depth with a random seed value of 3006. It caused the removal of 448 bacterial/archaeal (total amount of 9626 ASVs) and 283 fungal ASVs (total amount of 3709 ASVs) because they were no longer present in the dataset.

Sequence data was deposited in the Sequence Read Archive of NCBI and is available under accession number PRJNA646655.

Table 2 PCR Mastermix and reaction conditions for Amplicon Sequencing.

Gene	Primer (forward and reverse), Sequence & Reference	Mastermix	V [µl]	No. of Cycles	T [°C]	t
16s rRNA Bacteria & Archaea	Arch0519 F CAGCMGCCGCGGTAA Klindworth *et al.*, 2013	NebNext High Fidelity Mix	12.5	1	98	5 min
		F- & R primer	0.5		98	20 sec
	Pro 805 R GACTACNVGGGTATCTAATCC Herlemann *et al.*, 2011	BSA (3%)	1	25	51	20 sec
		DEPC water	9.5		72	30 sec
				1	72	5 min
ITS Fungi	ITS 3 mix CATCGATGAAGAACGCAG CAACGATGAAGAACGCAG CACCGATGAAGAACGCAG CATCGATGAAGAACGTAG CATCGATGAAGAACGTGG Tedersoo *et al.*, 2015	NebNext High Fidelity Mix	10	1	95	15 min
		F- & R primer	0.5		95	30 sec
		BSA (3%)	0	27	55	30 sec
		DEPC water	13		72	60 sec
				1	72	10 min
	ITS 4 mix TCCTCCGCTTATTGATATGC TCCTGCGCTTATTGATATGC TCCTCGCCTTATTGATATGC TCCTCCGCTGAWTAATATGC Tedersoo *et al.*, 2015					

2.3.5 Statistical data analysis

Experiment 1 - Biocrusts from forest soils

Data analysis of experiment 1 was performed with R Version 3.6.1 (R Core Team, 2019). Variances of abiotic and qPCR data of *experiment 1* were analyzed using linear mixed effect models (function lme in R package nlme (Bates and Pinheiro, 1998)) to disentangle biocrust

or exploratory effects. These models are particularly well suited for unbalanced sampling designs and consider multiple sampling within one plot with setting plot number as a random factor. The restricted log-likelihood was maximized to fit models. Models for variation analyses were verified by testing residual vs. fitted plots and sample quantile vs. theoretical quantile plots for normal distribution and homogenous variance of residuals. As conditions of homogeneity or normality were not met, data was transformed: *1+log* for abiotic parameters and gene abundances (except DON and P_{H2O}: *square root* transformation). Biocrust and bare soil samples were analyzed separately to unravel different effects in both compartments.

Furthermore, pairwise comparisons were performed by a Tukey Post hoc test with package lsmeans (Lenth, 2016) to detect significant ($p < 0.05$) differences caused by biocrust or exploratory effects. The ratio r was also defined to evaluate individual variations between biocrusts-bare soil pairs as r = biocrust/bare soil or as r = -bare soil/biocrust for bare soil > biocrust. An average ratio was calculated as the mean of all sampling spots for each variable within one exploratory.

Figures were created using the ggplot2 package Version 3.3.5 (Wickham, 2009). Pearson correlation plots were created using the qgraph package Version 1.9.2 (Epskamp *et al.*, 2012).

Experiment 2 – Biocrusts from agricultural soils

Data analysis of experiment 2 was performed with R Version 3.6.1 (R Core Team, 2019). For experiment 2, linear models were applied to disentangle biocrust or treatment effects. Models for variation analyses were verified by testing residual vs. fitted plots and sample quantile vs. theoretical quantile plots for normal distribution and homogenous variance of residuals. As conditions of homogeneity or normality were not met, data was transformed: *log* for soil parameters and gene abundances; *square root* transformation for taxa at the family level). Biocrust and bare soil samples were analyzed separately to unravel different effects in both compartments.

Rarefaction curves to evaluate sequencing coverage, alpha diversity (Pielou's evenness (Pielou, 1966), richness as the number of ASVs and diversity (Shannon diversity (Shannon, 1948)) were determined with vegan package Version 2.5-7 (Oksanen *et al.*, 2017) on subsampled data.

Variations in community composition on ASV level for Bacteria/Archaea and Fungi were analyzed based on Bray-Curtis-distance with PERMANOVA (Oksanen *et al.*, 2017), and variances on the family level were evaluated with linear models to disentangle biocrust or treatment effects.

Figures were created using the ggplot2 package Version 3.3.5 (Wickham, 2009).

Correlations for network analysis were calculated using Cytoscape Version 3.7.2 (Shannon *et al.*, 2003) with CoNet (Faust and Raes, 2016) as an add-on, where ASVs unspecified in order level or higher were removed prior to the calculations. Bacterial/Archaeal and fungal data were merged after separate computations of relative abundances. To reveal differences in co-occurrence patterns caused by sample and management type and to achieve a decent amount of replicates for the network computation, it was decided to disintegrate the full-factorial design and compute each 12 biocrust and bare soil samples together belonging to one management type. This results in the following networks to be calculated: biocrusts 120, bare soil 120, biocrusts 240, bare soil 240, biocrust -org, bare soil -org, biocrust +org, bare soil +org, biocrusts cT, bare soil cT, biocrusts rT, bare soil rT). ASVs must be present in 10 samples to validate correlations for each full-factorial variation. Pearson and Spearman correlations and Bray-Curtis and Kullback-Leibler dissimilarities were used to determine significant positive and negative interactions. Correlations need to be supported by two of those to be counted. Brown p-value merging gave 1000 permutations and bootstrap scores (Brown, 1975). The network visualization was performed in Gephi 0.9.2 (Bastian *et al.*, 2009) for undirected networks in the Fruchterman-Reingold layout (Fruchterman and Reingold, 1991). Figures showing family-level correlation partners were created using the function chordDiagram of circlize package Version 0.4.13 in R (Gu *et al.*, 2014). The highest connected nodes were defined as network hubs, which need to be specified to the family level to be considered (Tipton *et al.*, 2018).

3. Discussion

3.1. Disturbance creates opportunities for biocrust development

In (semi-)arid areas, the development of biocrusts and other vegetation is mainly limited by water, scarce nutrient contents, and high solar radiation (Gaur and Squires, 2017). As biocrusts developed strategies to cope with these challenging conditions, they displayed the dominant vegetation form and covered vast parts of the soil surface (Belnap *et al.*, 2001). Natural disturbances, such as burrowing animals, or anthropogenic impact, such as trampling, partially remove the biocrusts and set them back to initial developmental stages (Bu *et al.*, 2013; Ferrenberg *et al.*, 2015; Steven *et al.*, 2015). Due to the harsh conditions, the regeneration after disturbances - depending on the strength and type - takes very long and can result in years, or up to several decades, of recovery (Belnap *et al.*, 2016). Contrary to mesic regions, the favorable water and nutrient supply results in a highly competing phototrophic community, where small plants growing close to the soil are shaded by taller plants when succession or the growing season proceeds. In (semi-)arid regions, disturbances cause the removal of biocrusts with long regeneration times, while disturbances in mesic regions are necessary as they enable the minimum criteria for biocrust development: bare soil and sufficient light (Weber *et al.*, 2022).

On the one hand, the creation of bare soil is needed. On the other hand, bare soil needs to be kept free. Disturbances are created by disrupting or eliminating an upper layer of plants or litter. This can occur naturally by, e.g., digging animals or fallen trees or by human-induced disturbances such as trampling or using heavy machinery to harvest trees in forests or crops on agricultural fields. Hence, especially managed, mesic environments are characterized by high frequencies of human-induced disturbances. This often creates vast areas of bare soils which can potentially be colonized by biocrusts (**P1**), and thus, biocrusts have lately been discovered on agricultural fields (**P3**) (Nevins *et al.*, 2020) and in forests (**P2**) (Baumann *et al.*, 2017; Glaser *et al.*, 2021; Ngosong *et al.*, 2020).

Generally, the biocrusts developed on agricultural fields of the long-term agricultural research station (**P3**). They were mainly found in small grooves or holes and, thus, especially where the tractor drove, i.e., tractor tracks. Since these spots are supposed to have higher water contents, this could have supported the development of biocrusts (Dojani *et al.*, 2011). Increased biocrust cover has also been observed near organic material. It can be assumed that this dead plant debris is a resource for the biocrust community. The space between the rows of sugar beet was relatively wide, so bare soil was present throughout the season. This is hardly shaded as the crop growth is small and mainly grows belowground. Other crops like maize allow biocrust cover throughout the season (**P1**). On agricultural fields, a disturbance

occurs due to tilling the soil surface before planting in different intensities (**P3**). Conventional tillage includes plowing, which causes a turning of topsoil. Thus, any biocrust which might have been present from the previous growing season is removed. Despite this removal, a considerable cover of biocrusts was observed under conventional tillage at the end of the season before harvest (**P3**). As the chlorophyll *a* concentration of the biocrusts did not vary significantly between the different fertilization or tillage treatments, it can be assumed that an active and functional phototrophic biocrust community developed since the last management activities. On plowed fields and supposedly biocrust-free soil, an increased relative abundance of Cyanobacteria was found (**P3**). They are particularly typical for the initial stages of biocrusts, and therefore it can be assumed that the agricultural fields are in a continuous process of biocrust formation (Belnap *et al.*, 2016; Bu *et al.*, 2013; Ferrenberg *et al.*, 2015; Kuske *et al.*, 2012; Lange *et al.*, 1997; Maier *et al.*, 2018; Steven *et al.*, 2015). In contrast, no biocrusts could be collected on plots with minimal tillage, as grasses overgrew and covered bare soil. It is particularly interesting in this context that under reduced tillage, which only breaks up the soil but does not bury its old surface, both in biocrusts and in bare soils, much higher proportions of co-occurrences could be observed. Reduced tillage does not seem to destroy the community structure, and connections need not be established from scratch every year (**P3**). To summarize, while a certain level of disturbance is necessary for biocrusts to develop on agricultural soil surfaces, their community structure is significantly impacted if it exceeds a certain level. Whether this also impacts their functionality and soil stability needs to be investigated in future studies.

In forests, disturbance is also needed to create bare soil (**P2**). This occurs more randomly and varies widely compared to controlled agroecosystems. The surface disturbance is often naturally induced. In contrast to (semi-)arid areas, animals do not remove an existing biocrust cover but remove plants or litter and allow biocrusts to develop (Eldridge *et al.*, 2015). Naturally fallen trees often lift the root plate. This allows crusts to settle on the exposed subsoil and bare soil adhering to the root plate (Glaser, Albrecht, *et al.*, 2022). Human-induced surface disturbances in the forest are caused primarily by walking paths or skid trails of harvesters. Due to recurring trampling, it can be assumed that biocrusts would develop on walking paths only at the edges or rarely used paths, as is also known from arid areas (Kuske *et al.*, 2012). Harvester traffic, however, is much less frequent, so the bare soil caused by disturbance is predestined for developing biocrusts. Therefore, intense silvicultural management increases the chances of biocrust occurrence (**P1**). Regardless of the type of disturbance or management intensity, crusts were found on deciduous (beech, oak) and coniferous (spruce, pine) plots in all three exploratories. Thus, in mesic areas, they are not limited to sites with low nutrient contents or harsh conditions, such as dunes or former heaps (Glaser, Van, *et al.*,

2022; Sommer *et al.*, 2020). Disturbance of the soil surface allows them to develop in mesic areas precisely where human-induced management caused the creation of bare soil, regardless of the nutrient content or the parent material of their substrate (**P2**).

After soil surface disturbances, biocrusts developed within a few weeks and appeared as a transition stage in the succession process in mesic regions (Seitz *et al.*, 2017). Hence, they undergo a seasonal change with a peak of biomass in spring as pioneer communities (**P2**) (Ngosong *et al.*, 2020). Biocrust characteristics disappeared with the establishment of vascular plants within the biocrust (Gall *et al.*, 2022) or due to the shading from higher plants shading them during summer (**P1**). Thus, their development or continuation is disturbed or reduced. Later in the year, as with higher life forms, low temperatures lead to a reduction in metabolism, and snow in winter covers the surfaces and, with that, the biocrusts. However, if renewed disturbances occur, the way is paved for establishing new biocrusts (Szyja *et al.*, 2018) or allowing biocrusts to persist for several years (Seitz *et al.*, 2017).

This confirms the assumption that the recovery of biocrusts in mesic regions of managed sites is much faster compared to their (semi-) arid counterparts and takes place within a few months (**P1**, **P2**, **P3**) like, observed in Africa, shortly after the rainy season (Dojani *et al.*, 2011). Still, it is unclear if there is a delay in biocrust establishment at the beginning of the season due to increased disturbance levels, and further studies are needed to disentangle seasonal changes in biocrust biomass, community composition, and functional potentials throughout the year.

3.2. Biocrusts as hotspots for nutrients and microbial nutrient transformation in mesic, managed environments

Hotspots are defined as small loci in the soil with microbially mediated increased process rates and interactions with high-intensity interaction between nutrient pools (Kuzyakov, 2010; Kuzyakov and Blagodatskaya, 2015). Due to the limitation of carbon in most ecosystems, the availability of carbon immediately activates microbial metabolic processes (Hodge *et al.*, 2000; Kaštovská *et al.*, 2022). Therefore, the rhizosphere hotspot is induced by the priming of plants with the excretion of substances containing available carbon. In the detritusphere, this is achieved by releasing labile carbon due to organic matter degradation (Charlotte *et al.*, 2020), while also the drilosphere and other biopores are described as hotspots (Bauke *et al.*, 2017).

In biocrusts from agricultural soils, carbon pools were also found to be increased compared to bare soils (**P3**) (Kheirfam *et al.*, 2020). Moreover, labile carbon was correlated to the chlorophyll *a* content, which indicates the activity of photosynthesis performing organisms. Thus, photosynthesis in biocrusts can be understood as a hotspot-inducing priming effect like in other well-described hotspots (Charlotte *et al.*, 2020; Kuzyakov and Blagodatskaya, 2015; Pathan *et al.*, 2020; Spohn *et al.*, 2015). The increased value of chlorophyll *a* in the biocrusts compared to bare soil shows that photosynthesis occurs primarily in the biocrusts (**P3**). Since small amounts of chlorophyll *a* were also detected in bare soils, this can be understood as a precursor to biocrusts and, thus, to hotspot formation on agricultural fields (Kuzyakov and Blagodatskaya, 2015). Furthermore, labile nitrogen concentrations were increased in biocrusts compared to bare soils. Although nitrate was significantly increased by mineral fertilizer application, the values measured in the biocrusts were still higher than in the bare soils (Peng and Bruns, 2019). In nutrient-poor systems, nitrogen fixation by microorganisms is an important input path to explain increased nitrogen values (Kidron *et al.*, 2015). In well-developed systems like agricultural fields or forests, the high energy-demanding nitrogen fixation process is expected to be much less critical (Zehr *et al.*, 2003). This might also be traced back to the polymeric matrix of biocrusts, which can increase nutrient or humidity retention (Cania *et al.*, 2019; Costa *et al.*, 2018; Rossi *et al.*, 2018). Plantings could also benefit from these retained nutrients early in the growing season, although this would need to be investigated in future studies (**P3**). In addition, higher abundances of microbes were detected in biocrusts (Castillo-Monroy *et al.*, 2011; Glaser, Albrecht, *et al.*, 2022; Glaser, Van, *et al.*, 2022; Maier *et al.*, 2018; Nevins *et al.*, 2021). As especially Proteobacteria or Bacteroidetes were higher abundant in biocrusts than in bare soils, the increased biomass might be related to the increase of copiotrophic bacteria (Fierer *et al.*, 2007; Glaser, Albrecht, *et al.*, 2022). Also, in other hotspots, an increase in copiotrophs was detected, and at the same time, a

decrease in diversity was observed in some of the biocrusts of this experiment (Glaser, Albrecht, *et al.*, 2022; Spohn *et al.*, 2015).

The analysis of forest biocrusts not just revealed higher labile contents of nitrogen and phosphor but also the gene abundances compared to bare soils were higher for the mineralization of organic phosphorus (*phnX* und *phoD*) in all three exploratories (**P2**) (Marschner *et al.*, 2011; Richardson *et al.*, 2009; Spohn and Kuzyakov, 2013). Earlier studies of biocrusts from the exploratory Schorfheide did show reduced amounts of minerals but increased amounts of organic phosphorus compared to underlying bulk soil (Baumann *et al.*, 2017). Precisely the bacterial gene encoding enzymes responsible for the solubilization of mineral phosphorus (*gcd*) was significantly higher in the biocrusts of the Schorfheide than in the bare soil (**P2**). It was concluded that biocrust-inhabiting bacteria contribute to transforming mineral-bound phosphorus into organic substances and, beyond that, lead to increased transformation rates.

The only exception was the abundance of *phoD* in the Alb, the site with the highest nutrient contents (**P2**). Thus, even though biocrusts were found at all three sites, it is primarily on the soil surface of the nutrient-poor sites in Schorfheide (**P2**) and the low-quality agricultural soil (**P3**), where biocrusts form particularly well hotspots of microbial abundance and nutrient turnover, like observed from biocrusts of nutrient-poor systems with low aggregate stability like dunes or from other hotspots like rhizospheres (Brankatschk *et al.*, 2013; Cania *et al.*, 2019; Schulz *et al.*, 2016, 2013).

However, hotspots like the rhizosphere persist during the whole vegetation period, while for biocrusts, a limited existence was observed in the forest (P2) and agricultural fields (**P3**). In the forests, their existence is limited to spring, when it is already warm enough for growth but not yet shaded by trees - just as in fields of cereals with narrow rows. In fields with wide rows, like corn or sugar beets, they can persist until harvest but are destroyed or, at the very least, disturbed during harvest or tillage treatments (**P1**). Thus, they show accelerated but short-lived growth (**P2**, **P3**). However, they have also been found in lemon tree orchards, where plowing is not done yearly, and biocrusts persist for longer (Nevins *et al.*, 2020). Additionally, they are detected increasingly frequently in agricultural systems and, especially in managed sites in mesic areas with well-supplied soils – compared to deserts or dunes – still increase nutrient contents. Together with the fact that biocrusts as hotspots have an intensive nutrient turnover, their global influence on the biogeochemical cycles on managed sites might be highly underestimated (**P1**, **P2**, **P3**).

3.3. Biocrusts facilitate positive interactions among different taxonomic and functional groups

In the previous chapters, it was shown that the biocrusts sampled from mesic, managed sites show typical characteristics of biocrusts, as they are known from arid regions and, e.g., have increased nutrient concentrations, higher abundances of genes coding for nutrient turnover as well as higher abundances of microorganisms, like observed from microbial hotspots. From drylands or nutrient-poor areas, biocrusts are further known to be encapsulated by a polymeric matrix produced by the excretion of EPS from its members (Cania *et al.*, 2019; Rossi *et al.*, 2018). This matrix was shown to promote the interaction of microorganisms by trapping nutrients, improving cell movement or horizontal gene transfer (Costa *et al.*, 2018; Rossi *et al.*, 2018). Complex communities, like soils, have been explored to reveal the potential influences of different groups on each other with correlation analyses (Barberán *et al.*, 2012; Berry and Widder, 2014; Faust and Raes, 2016). On the one hand, positive correlations display co-occurrences where microorganisms might share the same niches or degrade substances in a cooperating cascade (Shi *et al.*, 2016; Velmourougane *et al.*, 2017; de Vries and Wallenstein, 2017). On the other hand, negative correlations display mutual exclusions as they could occur when taxa feed on the same nutrients, but one is more successful and suppresses the other (Kramer *et al.*, 2020).

In samples collected for this thesis, the positive correlations of the groups involved are higher in the biocrusts compared to bare soils (**P2**, **P3**). Therefore, it can be assumed that the microorganisms involved interact and profit from each other, as observed from other hotspots like the rhizosphere (Shi *et al.*, 2016). Particularly copiotrophic taxa were identified to be involved in the networks and enriched in networks of agricultural biocrusts (**P3**) as they benefit from high levels of easily available carbon sources (Pombubpa *et al.*, 2020; Shi *et al.*, 2016; Spohn *et al.*, 2015). Furthermore, the protective polymeric matrix of biocrusts reduces the impact of environmental stress, making it easier for microorganisms to exchange substrates (Lan *et al.*, 2010). In this regard, it is very interesting that the bacterial families involved in the formation of EPS in temperate biocrusts (**P2**) or former mining sites could be identified in the agricultural biocrusts (**P3**) as network hubs playing an essential role for the microbial community structure (Cania *et al.*, 2019; Vuko *et al.*, 2020). Hence, EPS production could be assumed to be a driver of community structure in mesic, managed biocrusts (**P3**). However, functional analysis on metagenomes or cultivation experiments must confirm this observation in future studies.

On fields with crop residues containing high amounts of organic material, even higher shares of co-occurrences in biocrusts could be detected (Ling *et al.*, 2016; Wang *et al.*, 2017). Since

the absolute abundance did not increase, an actual increase in the cooperation between microorganisms can be assumed. Furthermore, decomposers involved in chitin and cellulose degradation were found to be key taxa involved in organic matter decomposition (Banerjee *et al.*, 2016) and also detected as network hubs in the agricultural biocrusts (**P3**) (*Sphingomonadaceae*, *Burkholderiaceae*, *Chitinophagaceae*, *Nocardioidaceae*, and *Pleosporaceae*) (Coenye, 2014; Glaeser and Kämpfer, 2014; Maier *et al.*, 2018; Wieczorek *et al.*, 2019). It must be emphasized that the crop residues caused increased co-occurrences between Proteobacteria and Fungi, but solely in biocrusts (**P3**). Hence, biocrusts do not just promote the decomposition of organic material but also facilitate that Bacteria and Fungi interact across kingdoms, benefit and accomplish this together to obtain nutrients in parallel (Velmourougane *et al.*, 2017; de Vries and Wallenstein, 2017).

Since microbes aim to keep a stable, constrained ratio of carbon, nitrogen, and phosphorus in their cells (Cleveland and Liptzin, 2007), the critical role of decomposition in biocrusts suggests that nutrients are delivered to the same amount by the mineralization of organic material (Heuck and Spohn, 2016; Sorkau *et al.*, 2018). Like this, the functional analyses of biocrusts from mesic forests revealed strong correlations between nitrogen and phosphorus turnover (**P2**). This was detected in all samples, independent if from bare soil or biocrust, but especially pronounced where the concentrations of available nutrients were high (**P2**). Particularly interesting in this context is that the positive correlations between the nutrient pools and processes are only higher in biocrusts compared to bare soils in the exploratory with the lowest nutrient contents, in the sandy Schorfheide. Here, the abundance of the phosphonatases gene (*phnX*) was further higher than the one of phosphatases (*phoD*), which hints at lower availability of more easily degradable phosphates instead of more hardly degradable phosphonates (Bergkemper, Schöler, *et al.*, 2016). This result indicates that the availability of phosphorus is low in Schorfheide, forces bacteria to develop specific processes to break down hardly degradable substances, and might be one reason why the positive correlations are increased in this exploratories, to cooperate in breaking down organic material to make the most the little which is available. However, in the samples from the Alb, where the detected concentrations of nitrogen and phosphorus were highest, the abundances of *phoD* (phosphate mineralization) and *nifH* (nitrogen fixation) were not higher in biocrusts but in bare soil (**P2**). Thus, it can be assumed that the acquisition of phosphorus and nitrogen also occurs parallel in bare soils but is not closely linked, as observed from biocrusts.

4. Conclusion and Outlook

The investigation of biocrusts from varying mesic, managed sites across Germany allowed to analyze a wide range of regions and to perform a wide variety of nutrient and microbial analyses on a taxonomic as well as on a functional level to give insights into the structure and function of biocrusts depending on land use, like agricultural or silvicultural sites.

Taken the results together, it can be summarized that in mesic, managed regions, especially the sites with low nutrient contents or rather unfavorable conditions, like the exploratory Schorfheide or the agricultural experimental farm in Speyer, revealed typical biocrust characteristics in being a hotspot for available nutrients, increased amounts of microbial abundance, and nutrient transformation (H2). Furthermore, the results indicate a cooperative environment within the biocrusts, where EPS formation and organic matter degradation are key functions (H3). Nevertheless, biocrusts were also detected on well-developed sites with favorable conditions, like the exploratory Hainich or Alb, but with varying characteristics. Instead of establishing fully functional biocrusts, they selectively establish certain features that may positively contribute to their community.

However, surface disturbances are indispensable for creating bare soil and enabling biocrust development in mesic regions, where the final successional stage is dominated by higher plants that cover or shade the soil (H1). In these managed sites, the disturbances are mainly anthropogenic and recurrent, such as tillage or driving with harvesting machinery. If the disturbance is insufficient, as in the case of minimal tillage, lack of fertilizer, or forest management, the soil cover dominates, no bare soil exists, and biocrusts cannot establish. However, future studies must clarify which factors influence the development of mesic biocrusts in detail. It can be assumed that a certain level or frequency of disturbance must not be exceeded. Otherwise, the incipient biocrust growth will be interrupted again. In addition, it would be essential to investigate the early stages of biocrust growth and their establishment. Within this thesis, cyanobacteria and chlorophyll *a* were also detected in the bare soil of the arable soils, so it can be assumed that bare soil is always in a pre-crust stage. It remains unclear whether the observations made would be confirmed in subsequent years, how the development changes during one season, and how extreme events such as heavy rain or dry summer periods would influence the persistence of biocrusts. Such studies are particularly relevant given the climate change, as these extreme weather events will occur more frequently in the latitudes of mesic regions. Future studies should also be directed at the extent and persistence of biocrust cover to more precisely assess their influence on (global) biogeochemical cycles.

Furthermore, it could be shown that biocrusts can increase the available nutrient concentrations and moisture compared to bare soil. However, the question remains to what extent these are available to organisms outside the biocrusts and can, for example, favor the growth of arable plants during their establishment. In this context, it would also be interesting to evaluate how a change in gene abundances on the DNA level transfers to activity on the RNA level and how this impacts microbial activity and actual turnover rates.

In the frame of this research project, very extensive investigations have been carried out on agricultural land characterized by a low quality for crop growth and during the vegetation of sugar beets. The question remains whether, similar to the Alb and Hainich, they would also establish well on arable soils of good quality and how the establishment and duration of biocrusts proceed under other arable crops. This would also be highly relevant for global classification, as the diversity of agricultural and forestry soils and planted crops exceeds the one investigated during this thesis.

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Appendices

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VIEWPOINT

Biocrusts: Overlooked hotspots of managed soils in mesic environments

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Abstract

Biological soil crusts, or "biocrusts", are biogeochemical hotspots that can significantly influence ecosystem processes in arid environments. Although they can cover large areas, particularly in managed sites with frequent anthropogenic disturbance, their importance in mesic environments is not well understood. As in arid regions, biocrusts in mesic environments can significantly influence nutrient cycling, soil stabilization, and water balance; however, their persistence may differ. We call for interdisciplinary physical, biological, microbiological, chemical, and applied soil science research with a special focus on biocrusts of managed soils from mesic environments, to better understand their impact on overall ecosystem health and resilience, particularly with regard to climate change.

KEYWORDS
biological soil crust, climate change, erosion, hotspot, managed sites, mesic environments, soil degradation

1 | OVERLOOKED BIOCRUST HABITATS

Biological soil crusts (hereafter referred to as biocrusts) are hotspots of microbial activity, characterized by large amounts of microbial biomass, high nutrient turnover rates, and intensive biotic interactions. This is due to the supply of numerous bioavailable organic compounds provided by plants and/or animals (Kuzyakov & Blagodatskaya, 2015). Biocrusts develop on and a few millimeters below the soil's surface, and modify their surroundings with organismal metabolites to create new habitats. Typical biocrust biota include algae, cyanobacteria, fungi, bacteria, archaea, protists, lichens, bryophytes, and microarthropods (Belnap et al., 2001; Khanipour Roshan et al., 2021; Weber et al., 2016, 2022). Biocrusts play an important ecological role in the creation and maintenance of healthy soils, and can (1) improve nutrient availability and fertility (Evans & Ehleringer, 1993; Gao et al., 2010; Li et al., 2012), (2) influence plant germination (Godínez-Alvarez et al., 2012; Havrilla et al., 2019; Zhang & Belnap, 2015), (3) increase biogeochemical cycling (Miralles et al., 2012; Wang et al., 2017; Xu et al., 2013), (4) keep and enhance water availability at the soil surface (George et al., 2003; Li et al., 2022), (5) increase soil aggregate stability (Cania et al., 2020; Riveras-Muñoz et al., 2022; Zhang et al., 2006), and (6) protect the soil surface by counteracting soil erosion from water (Chamizo et al., 2017; Seitz et al., 2017) or wind (Bullard et al., 2022; Zhang et al., 2006). However, thus far, biocrusts have

primarily been studied in arid and semiarid regions (Weber et al., 2016).

Most studies of biocrusts in temperate regions have concentrated on bare soils or on soils with minimal vascular plant cover. Similar to arid soils, these soils are often too poor for vascular plant establishment and growth, with high salinity and/or low nutrient and water availability (Corbin & Thiet, 2020). Some temperate regions that biocrusts have been investigated include coastal areas (Khanipour Roshan et al., 2021; Mikhailyuk et al., 2019; Schulz et al., 2016; Thiet et al., 2014), inland dunes (Fischer, Veste, Wiehe, et al., 2010; Thiet et al., 2005), sand plains and pine barrens (Gilbert & Corbin, 2019; Hawkes & Flechtner, 2002), reclaimed lignite open-cast mining sites (Fischer, Veste, Schaaf, et al., 2010; Gypser et al., 2015), and potash tailings piles (Pushkareva et al., 2021; Sommer et al., 2020). Corbin and Thiet (2020) focused their review on biocrusts in temperate environments with restricted vascular plant productivity due to challenging soil and/or climatic conditions. While low vascular plant cover is common in arid regions, that is not reflective of most temperate regions. These regions are largely characterized by adequate water availability and unrestricted vascular plant growth, which can also be colonized by biocrusts. Recent studies have also found biocrusts at mesic, managed sites, which are anthropogenically impacted, such as monospecific pine forests, broadleaf-mixed forests, and agricultural fields (Baumann et al., 2017; Gall et al., 2022; Glaser et al., 2018; Kurth et al., 2021; Nevins et al., 2020, 2021; Ngosong et al., 2020). As the study of biocrusts on managed soils in mesic environments is still in its infancy, herein, we will elaborate on their dynamics, distribution, and potential impacts on ecosystem services.

2 | BIOCRUST DEVELEOPMENT ON DISTURBED SILVI- AND AGRICULTURAL SOIL SURFACES IN MESIC ENVIRONMENTS

The essential requirements for biocrust development include bare soil and a minimum amount of light. These conditions act as a starting point for biocrust establishment and succession, and can be created in mesic environments by disturbing or removing layers of vegetation and/or litter. As a result, soil is directly exposed to sunlight and biocrusts can rapidly colonize within a few weeks (Seitz et al., 2017). Recent work has described biocrusts in forests (Baumann et al., 2017; Gall et al., 2022; Glaser et al., 2018; Kurth et al., 2021; Ngosong et al., 2020) and on agricultural fields (Nevins et al., 2020, 2021, 2022). In these environments, biocrusts are ephemeral and do not usually persist unless the disturbance is permanent (Szyja et al., 2018).

In forests, bare soil can be natural or human induced. The total area of natural (e.g., caused by pest insects, disease, heavy storms, drought stress) and anthropogenic (e.g., clearcutting, forest roads, or skid trails) disturbance amounts to 39 million hectares, or 17% of the total area of all European forests (Senf & Seidl, 2021). Biocrusts can be found in both coniferous and deciduous forests of mesic environments, and are visible in the field as green cover (Baumann et al., 2017; Glaser, Albrecht, et al., 2022; Kurth et al., 2021) (Figure 1). While they can quickly establish in disturbed areas such as skid trails, their biocrust characteristics rapidly disappear with succession of vascular vegetation (Gall et al., 2022). Other cryptogamic communities that host a large part of their biomass above the soil's surface (such as thick moss mats, which are common in coniferous forests) are not always classified as biocrusts. However, there is a smooth transition between these communities and biocrusts (Belnap et al., 2003; Weber et al., 2022).

Biocrusts have also been found on agricultural soils (Figure 1), often in conjunction with copiotrophic microorganisms (Nevins et al., 2020, 2021, 2022). Agricultural practices such as plowing or other methods of tillage create large amounts of bare soil. This bare soil provides niches for biocrust development until crops shade the ground (limiting the light required for biocrust development). Additionally, many crops, such as potatoes, sugar beet, and maize, are grown in rows that allow for solar radiation to reach the ground during the entire growing season. In Europe, this results in 12.4 million hectares of potential biocrust cover, or approximately 12.6% of total arable land (Eurostat, 2020).

As biocrusts have been documented in forests and agricultural fields, they have the potential to colonize very large areas in mesic environments. Considering this and the fact that biocrusts are biogeochemical hotspots that can increase nutrient pools and turnover rates (Glaser et al., 2018; Kurth et al., 2021; Nevins et al., 2020), we hypothesize that they play a significant role in nutrient cycling in agri- and silvicultural soils, but this perspective has not yet been addressed.

3 | BENEFICIAL EFFECTS OF BIOCRUSTS IN MESIC ENVIRONMENTS

A large number of beneficial ecosystem functions can be attributed to biocrust development (Weber et al., 2016). However, there are very few studies dealing with the beneficial effects of biocrusts in mesic environments, and even fewer address managed soils.

In disturbed areas, biocrusts have great potential to reduce soil erosion (Seitz et al., 2017), and in some cases are even more effective than vascular plant cover (Bu et al., 2015; Gall et al., 2022). In particular, pioneer biocrust cover can protect against erosion as early as a few weeks following timber harvest (Gall et al., 2022), a very vulnerable stage for soils. Three main erosion-reducing mechanisms in biocrusts have been described. First, the sticky filamentous structure of many pioneer microalgae and cyanobacteria can glue soil particles together (Glaser et al., 2018; Glaser, Albrecht, et al., 2022; Glaser, Van, et al., 2022). Second, biocrusts are able to store water and reduce the kinetic energy of raindrops relative to bare soil (Zhao et al., 2014), which can reduce overland runoff (Bu et al., 2015). Third, biocrusts can increase soil organic matter (Gao et al., 2017) and improve aggregate stability by bacterial metabolites such as exo- and lipopolysaccharides (Cania et al., 2020). However, these effects depend on climatic conditions (Kidron, Lichner, et al., 2022; Riveras-Muñoz et al., 2022) and species composition (Gypser et al., 2016) and have been poorly studied in mesic environments. As shown in Kidron, Lichner, et al. (2022), biocrust-related mechanisms of runoff generation are very complex, with significant variability documented in arid environments.

FIGURE 1 Overview of biocrusts on managed soils in mesic environments: (A, B) early successional bryophyte-dominated biocrusts on skid trail wheel tracks in a deciduous forest; (C) bryophyte-dominated biocrust under leaf litter; (D) bryophyte- and cyanobacteria-dominated biocrusts on arable land between sugar beet crops

Increased surface runoff from biocrusts, for example, could lead to more soil erosion downslope, assuming an uncovered soil there. For a better understanding of biocrust-related mechanisms of soil erosion and runoff generation in mesic environments, more field experiments are necessarily needed.

The impact of biocrusts on the soil water balance in arid environments has been contradictory (Kidron, Fischer, et al., 2022; Kidron, Lichner, et al., 2022). On one hand, they can improve infiltration into the soil and increase water content while reducing evaporation—although these effects can vary depending on rainfall intensity, temperature, and soil texture (Chamizo et al., 2016). On the other hand, biocrusts may have a negative effect on the soil water balance, due to pore clogging by exopolysaccharides and/or water repellence (Kidron, Lichner, et al., 2022; Xiao et al., 2019). Additionally, recent studies of biocrusts in temperate environments have primarily been conducted in challenging conditions for vascular plant growth (Gypser et al., 2016; Thiet et al., 2005), and cannot be generalized. Therefore, further studies in managed mesic environments are needed to fully characterize the potential beneficial effects of biocrusts on the soil water balance.

Biocrusts have been referred to as biogeochemical hotspots in mesic environments (Kuzyakov & Blagodatskaya, 2015). They host higher microbial biomass compared to surrounding bulk soil (Glaser, Albrecht, et al., 2022; Glaser, Van, et al., 2022; Kurth et al., 2021; Nevins et al., 2021), exhibit more nutrient turnover, and can consequently impact biogeochemical cycling (Glaser et al., 2018; Kurth et al., 2021). Recent work has found a carbon enrichment from microbial biomass and plant-available nitrogen beneath biocrusts in agricultural soils (Nevins et al., 2020), and that biocrusts play a key role in the biogeochemical phosphorus cycle in forests (Baumann et al., 2017, 2019; Kurth et al., 2021). Artificially cultivated biocrusts have also been found to increase carbon, nitrogen, and phosphorus contents at the soil's surface (Deng et al., 2020; Wu et al., 2013). Kheirfam (2020) observed an increase in carbon sequestration when soils were inoculated with bacteria, cyanobacteria, or both, resulting in an extrapolated removal of 3.11–3.93 t ha^{-1} y^{-1} of CO_2 from the atmosphere. Several other studies have primarily been concerned with the composition of biocrust soil microbial communities (Glaser, Albrecht, et al., 2022; Glaser, Van, et al., 2022; Kurth et al., 2021; Nevins et al., 2021), and their changes with elevation and microclimates (You et al., 2021). However, further work

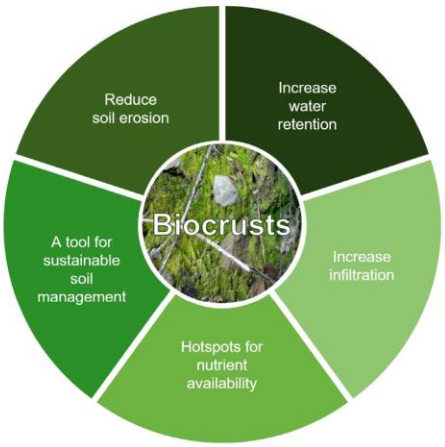

FIGURE 2 Summary of the potential beneficial effects of biocrusts in mesic environments (Illustration: Julia Dartsch)

will be required to determine which specific organisms or community profiles contribute to these changes in biogeochemical cycling. Additionally, future investigations could determine biocrusts' capability to store nitrogen or phosphorus temporally in their biomass, particularly over winter when microbial activity is reduced.

Based on these ecological functions, biocrusts bear the potential as novel tools for sustainable soil management. They have already been explored as possible avenues for the restoration of degraded soils, such as in the rehabilitation of salt heaps (Sommer et al., 2020) and felled/burned forests (Chamizo et al., 2020; Olarra, 2012). In addition to habitat restoration by loose soil particle stabilization (Grover et al., 2020), they can also serve as a "living" fertilizer in agriculture, as they biologically fix atmospheric nitrogen and retain nutrients and water (Sears & Prithiviraj, 2012; Vinoth et al., 2020). Methods to facilitate and accelerate biocrust establishment have primarily been applied in arid environments, and include the addition of chemical or physical soil stabilizers (Antoninka et al., 2020), improved light conditions (Zhao et al., 2021), irrigation (Wu et al., 2013; Zhou et al., 2020), and the inoculation of pioneer organisms with single or multispecies biocrusts to close gaps in natural biocrust cover (Bowker, 2007). In agriculture in particular, large-scale biocrust inoculation could be carried out by airplane in the future (Sears & Prithiviraj, 2012). We propose these approaches could also be applied for use in mesic environments after modification (Figure 2).

4 | OUTLOOK: BIOCRUSTS' POTENTIAL TO MITIGATE CLIMATE CHANGE IN MESIC ENVIRONMENTS

Global climate change is becoming increasingly visible in mesic environments, and will bring extreme weather events like heavy rain and extended drought (Olsson et al., 2019). As a result, soils will be more vulnerable and require new forms of management for their protection, as stipulated by the UN's "Sustainable Development Goals". Accordingly, biocrusts could make a significant contribution. Considering the large extent of biocrust colonization in managed mesic environments, and these areas' projected expansion due to climate change (Gejdoš & Michajlová, 2022; Senf & Seidl, 2021), further studies will be necessary to evaluate their contributions to ecosystem services and global relevance (Ferrenberg et al., 2017). Interdisciplinary physical, biological, microbiological, chemical, and applied soil research will be indispensable in understanding the development and influence of biocrusts in mesic and anthropogenically impacted environments. Their inoculation as an erosion control measure may be of particular importance (Cruz de Carvalho et al., 2018; Varela et al., 2021), especially as erosion rates are projected to increase due to climate change (Li & Fang, 2016). In addition, biocrusts' ability to store carbon could help in combating climate change in general (Kheirfam, 2020; Kheirfam et al., 2017), and applied in agriculture (Vinoth et al., 2020) or restoration (Román et al., 2018). We call for interdisciplinary research with a focus on biocrusts of managed soils in mesic environments, in order to better understand their multitrophic interactions, consequences on chemical and physical soil properties, and impact on overall ecosystem health.

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Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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B Publication 2

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ORIGINAL PAPER

Correlation of the abundance of bacteria catalyzing phosphorus and nitrogen turnover in biological soil crusts of temperate forests of Germany

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Abstract
Soil P pools are strongly driven by microbial activities, and vice versa, P pools shape bacterial communities and their functional potential. Biological soil crusts (biocrusts) represent a microbial hotspot for nutrient turnover. We compared biocrusts and bulk soil samples from different temperate beech (*Fagus sylvatica* L.) forests representing a gradient in soil texture, nutrient concentrations, and pH values at biocrust peak biomass. We measured the total and plant-available P and N concentrations and assessed the bacterial potential to mineralize (*phoD*, *phnX*), solubilize (*gcd*), and take up P (*pstS* and *pitA*) and mineralize (*chiA*, *apr*) and fix N (*nifH*) by quantifying the respective marker genes (qPCR). We found an increase of absolute and relative bacterial abundance involved in P turnover in biocrusts, but the strategy to acquire P differed between the regions as bacteria harboring the starvation-induced *pstS* gene were most abundant where labile P was lowest. In contrast, the region with lowest total P concentrations has a higher potential to utilize more stable phosphonates. N mineralization was strongly correlated to P turnover at regions with increased labile N and P concentrations. Interestingly, the potential to fix N was highest in the bulk soil where total P concentrations were highest. Even though the correlation of N and P turnover is strongest if their ratio is low, the acquisition strategy strongly depends on soil properties.

Keywords Biological soil crust · Microbial N turnover · Microbial P turnover · Temperate forest · qPCR · Biodiversity Exploratories

Introduction

Many terrestrial habitats are limited in the major nutrients P and N (Elser et al. 2007). The P concentration of the parent rock material, the turnover of the internally bound organic P, and the sorption of P onto soil particles most importantly determine the P availability in forest soils, as fertilizer hardly plays a role (Walker and Syers 1976). Changes in P pools are strongly driven by microbial activities and dependent on microbial community composition and its activity pattern (Richardson and Simpson 2011; Rodríguez et al. 2006). Vice versa, the composition of P pools in soil shapes microbial communities and determines their functional potential (Bergkemper et al. 2016a). However, not only P pools and P availabilities drive P transformation processes, but also the overall nutrient stoichiometry strongly influences microbial P turnover, as microorganisms keep a stable ratio of macronutrients in their biomass (Cleveland and Liptzin 2007). For example, Sorkau et al. (2018) described a positive correlation of microbial P and N in soils of different temperate forest regions. Moreover, a positive correlation of bioavailable P fractions in soil and microbes potentially able to fix N was found (Bergkemper et al. 2016a). Vice versa, inorganic P (P_i) limitation can repress N assimilation as the respective genes

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are under the control of the *Pho* regulon, which contains several genes controlled by a two-component system, which detects P_i concentrations in the environment (Santos-Beneit 2015). Highest turnover rates for plant-available nutrients like N, P, or C have been described for biological hotspots like rhizosphere or drilosphere in soil (Hoang et al. 2016; Kuzyakov and Blagodatskaya 2015; Lipiec et al. 2016; Reinhold-Hurek et al. 2015; Schulz et al. 2013; Uksa et al. 2015). Biological soil crusts (biocrusts) can be considered as such hotspots. Biocrusts are mostly dominated by organisms like phototrophic cyanobacteria, microalgae, lichens, or mosses, which drive the input of nutrients into the system. Together with associated heterotrophic microorganisms such as archaea, bacteria, or fungi, they create stable microhabitats, for example, by the excretion of polysaccharides (Cania et al. 2020; Mugnai et al. 2018, Vuko et al. 2020). Biocrusts have been mostly studied in arid and nutrient-poor habitats (Belnap et al. 2001), where they are the predominant vegetation form and their growth is limited by water and nutrient availability. Biocrusts from those regions contribute about half of the terrestrial N_2 fixation (Elbert et al. 2012). Moreover, it was demonstrated by Beraldi-Campesi et al. (2009) that biocrusts are not only enriched in N but that this also comes along with higher total P concentrations underlining the importance of balanced nutrient concentrations as proposed by Cleveland and Liptzin (2007). Less is known and only a few studies exist, which describe biocrusts as hotspots for nutrient turnover in temperate regions (Baumann et al. 2019; Brankatschk et al. 2013; Corbin and Thiet 2020; Gypser et al. 2016; Schaub et al. 2019; Schulz et al. 2016; Szyja et al. 2018), especially within well-developed ecosystems like forests (Baumann et al. 2017; Glaser et al. 2018; Williams et al. 2016). Atmospheric P inputs by dry and wet deposition in forests contribute as well since forests are dust traps also for particles from irrigated, highly fertilized agricultural soils (Aciego et al. 2017; Berthold et al. 2019) and those might be entrapped in the polymeric matrix of biocrusts. However, the relative contribution to P pools strongly depends on the bulk soil P concentration (Aciego et al. 2017). The role of biocrusts in P transformation in temperate forests has been described by Baumann et al. (2017), who found that, similar to biocrusts from arid regions, the P concentrations in biocrusts are enriched compared with adjacent bulks soils. Additionally, they demonstrated that especially the concentration of P-containing minerals decreased and of organic P concentrations increased in biocrusts compared with bulk soil. Thus, we hypothesize that (i) microorganisms colonizing biocrusts are involved in solubilization of mineral P and its transformation to biomass and thus abundances of those microorganisms are higher in biocrusts compared with bulk soil. (ii) As it was supposed that P and N turnover are closely linked, we further hypothesize that similar to P mineralization, also the potential for N mineralization is more pronounced in biocrusts, because of the higher microbial abundance, while the energy demanding fixation of N is less important in biocrusts from nutrient-rich forest soils. (iii) The strength of correlations between N and P turnover strongly depends on the N/P ratio of the bulk soil.

To test these hypotheses, we compared biocrust and bulk soil samples from temperate beech (*Fagus sylvatica* L.) forests, along a gradient in soil texture, nutrient concentrations, and pH values. Samples were taken once when biomass of biocrusts was highest. As the lifetime of biocrusts in temperate forests is short (they typically occur only at the end of the winter period before trees develop leaves) and the size of biocrusts is small as they newly develop every season, which excludes repeated sampling of the same biocrust, we abstained from repeated samplings but instead increased the number of analyzed replicates. Bacterial genes coding for proteins catalyzing P mineralization (alkaline phosphatase—*phoD*; phosphonoacetaldehyde hydrolase—*phnX*), solubilization (quinoprotein glucose dehydrogenase—*gcd*), and uptake (substrate binding protein of the phosphate ABC transporter—*pstS*; low-affinity Pi transporter—*pitA*) as well as N mineralization (bacterial chitinase group A—*chiA*; alkaline metalloprotease—*apr*) and N_2 fixation (dinitrogenase reductase subunit of the nitrogenase—*nifH*) were used as proxies for the abundance of the respective functional bacterial groups. We used qPCR to measure the abundance of bacterial genes involved in P and N turnover and correlated the data with the stable and labile P and N pools.

Material and methods

Sampling regions and procedure

All sampled forest sites are part of the Biodiversity Exploratories, a platform for interdisciplinary biodiversity research in Germany, with sites located in the southwest (Schwäbische Alb (ALB)), central (Hainich (HAI)), and northeast (Schorfheide-Chorin (SCH)) part of Germany (Fischer et al. 2010, www.biodiversity-exploratories.de). The three experimental regions differ gradually from south to north in altitude (ALB: 758 m, HAI: 415 m, SCH: 68 m), mean annual precipitation (year 2018, ALB: 806 mm, HAI: 357 mm, SCH: 539 mm), soil texture (silty clay in ALB and HAI, loamy sand in SCH), pH (ALB: pH 5.7 ± 0.8, HAI: pH 5.8 ± 1.2, SCH: pH 4.9 ± 1.5), and nutrient status (Alt et al. 2011; Grüneberg et al. 2010). The size of the regions ranges from 420 to 1300 km^2 and each exploratory includes 50 grassland and 50 forest plots with differing land use intensity.

Samples were taken in spring 2018 before bud break from 3 to 4 plots per region focusing on managed beech (*Fagus sylvatica* L.) forest plots with an even-aged tree population. At each plot, biocrust and bulk soil samples were collected from

skid trails at one to three spots, depending on their prevalence. Overall, 27 biocrust samples, visually recognized as green cover on the ground, were collected (see Fig. 1 and Supplement Table 1 for original plot numbers and number of biocrusts sampled). Biocrust samples (approx. 10 cm in diameter and 5 mm in thickness) were taken with a sterile Petri dish and transported on ice to the lab. As control, we sampled biocrust-free bulk soil (0–5 mm depth) close to the biocrust (max. distance of approx. 1 m), resulting in 54 samples in total. Samples for nutrient analyses were frozen at − 20 °C and samples for microbial analysis at − 80 °C until further analysis.

Abiotic soil properties

The pH was measured using 2 g of fresh sample material after 15 min shaking in 20 ml of a 0.01 M $CaCl_2$ solution. The water content was determined gravimetrically by the loss of weight after drying (60 °C, 24–48 h). The dried material was further used for phosphate and N_{total} analyses.

N_{total} was measured using approx. 30 mg of dried sample material in a CNS-Analyzer (vario EL, Elementar, Germany). The more alkaline soil samples of ALB were treated with 10% HCl and subsequently dried at 60 °C to remove carbonates prior measurement.

For the measurement of inorganic N compounds (exchangeable NH_4^+–N and NO_3^--N) as well as total dissolved nitrogen (TDN), 0.5 g material was extracted using 20 ml of a 0.01 mol l^{-1} $CaCl_2$ solution. The extracts were then filtered and stored frozen until analysis. The two inorganic fractions were directly analyzed using a constant flow analyzer (Flowsys, Alliance Instruments, Austria). For TDN, an aliquot of 10-ml extraction filtrate was digested according to Berthold et al. (2015) with peroxydisulfate for 24 h at 90 °C. The samples were neutralized after digestion and measured for NO_3^--N concentration like described above. The values for dissolved organic nitrogen (DON) were calculated by the subtraction of summed inorganic N fractions from the total dissolved N values.

All measurements of inorganic phosphate (after extraction or digestion) were carried out using the molybdenum blue photometric method (Hansen and Koroleff 1999). The absorbance was measured at 885 nm in a 5-cm optical glass cuvette using a photometer (UV1200, Shimadzu, Japan). Two easily available inorganic phosphate fractions of the Hedley fractionation (Hedley et al. 1982) were sequentially analyzed for H_2O-soluble phosphate (P_{H2O}) and $NaHCO_3$-fraction (P_{NaHCO3}) using approx. 1.5 g dried material. For total P (P_{total}) analysis, approx. 100 mg dried material was digested according to Berthold et al. (2015) for 24 h at 90 °C using an acid peroxydisulfate solution and neutralized before measurement.

DNA extraction and quantification

Phenol chloroform extraction of nucleic acids was performed with approx. 0.5 g of sample material according to Töwe et al. (2011) or without added material for extraction blanks, which served as extraction control (four processed controls). For homogenization, Lysing Matrix Tubes E (MP Biomedicals, USA) and the Precellys24 Instrument (Bertin Technologies, France) were used. The purity of the extract was checked by measuring the ratios of adsorption of 260 nm/280 nm and 260 nm/230 nm as given by NanoDrop® ND-1000 spectrophotometer (Thermo Fisher Scientific, USA). Yields of extracted DNA were quantified using Quant-IT™ Picogreen® dsDNA Assay Kit (Thermo Fisher Scientific, USA). The DNA concentration of the four extraction controls was below detection limit, and thus, DNA contamination during the extraction could be excluded.

Real-time quantitative PCR

A SYBR Green® based approach was applied by using a 7300 real-time qPCR machine (Applied Biosystems, Germany) to quantify gene abundances according to the manufacture's protocol. The reaction mix contained 12.5 µl of SYBR Green® (Thermo Fisher Scientific, USA), forward (F) and reverse (R) primers (Metabion, Germany), 0.5 µl BSA (3%, Sigma, Germany), and DEPC-treated water and

Fig. 1 Biological soil crusts in skid trails of region. **a** ALB, **b** HAI, **c** SCH

was set to 25 µl. Reaction conditions, primers, calibration standards, and full names of the chosen marker genes are summarized in Table 1. To exclude inhibitory effects, dilution tests were performed prior running qPCRs. Standard series ($r^2 > 0.99$), no template controls and samples diluted to 1/64 were included in each run. To evaluate the quality of the qPCR, melting curve analyses were performed and randomly chosen samples were checked by electrophoresis on a 1.5% agarose gel. The qPCR efficiency was calculated for each primer pair with the formula $Eff_{slope} = 10\text{^}(-1/slope) - 1$ and was in all measurements between 74 and 90%. Values below detection limit of 10 copies (according to manufacturer's protocol) per reaction were set NA.

Statistical analysis

Data analysis was performed in R version 3.6.1 (R Core Team 2019). Linear mixed effect models (function lme in R package nlme (Bates and Pinheiro 1998)), which are particularly well suited for unbalanced sample designs, were used to investigate variances caused by the region (ALB, HAI, SCH) and sample type (biocrust, bulk soil). Plot number was set as random factor to consider multiple sampling within one plot. Models were fit by maximizing the restricted log-likelihood. To validate the linear mixed effects models, residual vs. fitted plots and plots showing sample quantiles vs. theoretical quantiles based on the model were tested for homogenous variance and normal distribution of residuals. If either conditions of normality were not met or homogeneity of variance had to be improved, data was log transformed, except DON and P_{H2O} that were square root transformed. To test for significant differences ($p < 0.05$) between the investigated factors, pairwise comparisons were conducted by a Tukey post hoc test (R package lsmeans) (Lenth 2016). The dot and crossbar plots were created using the ggplot2 package (Wickham 2016); for Pearson correlation plots, the qgraph package (Epskamp et al. 2012) was chosen.

To see how abiotic properties and gene abundances differ on average between biocrust and bulk soil samples, we defined the ratio r, which is based on the mean of biocrust and bulk soil for each variable. In case the values of biocrust were higher than bulk soil values, this was calculated as $r =$ biocrust/bulk soil, else as $r = -$bulk soil/biocrust.

Results

Soil chemical properties

Soil chemical properties are shown in Fig. 2 and results of statistical analysis are summarized in Tables 2 and 3. The differences between the values of each pair of biocrust and bulk soil are shown in Supplement Fig. 1. N_{total}, P_{total}, and NO_3^--N concentrations and pH increased significantly ($p \leq 0.001$) in biocrusts and bulk soil to the same extent from SCH (N_{total}

Table 1 qPCR reaction conditions and calibration standards for qPCR of functional genes

Protein	Target gene	Reaction conditions				Calibration standard source
		F- and R-primer (pmol µl^{-1})	Thermal profile[a]	Primer	Reference	
Dinitrogenase reductase subunit of the nitrogenase	*nifH*	0.5	45 s/95 °C, 45 s/55 °C, 45 s/72 °C	nifH-f and -r	Rösch et al. (2002)	*Sinorhizobium meliloti* 30136
Alkaline metalloprotease	*apr*	1	45 s/95 °C, 45 s/53 °C, 45 s/72 °C	apr-f, apr-r	Bach et al. (2001)	*Pseudomonas aeruginosa* 5071
Bacterial chitinase group A	*chiA*	1	30 s/95 °C, 30 s/60 °C, 60 s/72 °C	chiA-f, chiA-r	Xiao et al. (2005)	*Streptomyces griseus*
16S ribosomal RNA	16S rRNA gene	0.5	45 s/94 °C, 45 s/58 °C, 45 s/72 °C	FP and RP 16S	Bach et al. (2002)	*Pseudomonas putida*
Alkaline phosphatase	*phoD*	0.8	20 s/95 °C, 60 s/60 °C, 30 s/72 °C, 60 s/81 °C	phoD-F, phoD-R	Bergkemper et al. (2016b)	*Bradyrhizobium japonicum*
Phosphonoacetaldehyde hydrolase	*phnX*			phnX-F, phnX-R		*Salmonella enterica* DSM 17058 (DSMZ)
Quinoprotein glucose dehydrogenase	*gcd*			gcd-F, gcd-R		*Salmonella enterica* DSM 17058 (DSMZ)
Phosphate inorganic transporter	*pitA*			pitA-F, pitA-R		*Pseudomonas fluorescens*
Phosphate-specific transporter (periplasmic phosphate-binding protein)	*pstS*			pstS-F, pstS-R		*Bradyrhizobium japonicum*

[a] Performed in 40 cycles

2289 ± 919 mg kg^{-1} dw; P_{total} 149 ± 47 mg kg^{-1} dw; NO_3^--N 7.26 ± 5.5 mg kg^{-1} dw) over HAI (N_{total} 3782 ± 768 mg kg^{-1} dw; P_{total} 327 ± 114 mg kg^{-1} dw; NO_3^--N 12.88 ± 5.8 mg kg^{-1} dw) to ALB (N_{total} 4588 ± 1402 mg kg^{-1} dw; P_{total} 624 ± 263 mg kg^{-1} dw; NO_3^--N 18.09 ± 7.7 mg kg^{-1} dw)). The same trend was observed for TDN, which spanned a range from ALB to SCH from 37.70 ± 8.74 mg kg^{-1} dw to 25.74 ± 9.15 mg kg^{-1} dw. In contrast, P_{NaHCO3} values were lower in HAI (9.79 ± 2.33 mg kg^{-1} dw) and SCH (12.48 ± 3.42 mg kg^{-1} dw) than in ALB (31.96 ± 21.14 mg kg^{-1} dw). Interestingly, P_{H2O} (11.2 ± 8.0 mg kg^{-1} dw) was detected at highest concentrations in SCH and DON was on the same level for all regions even though N_{total} and P_{total} were lowest.

Differences between biocrusts and bulk soil for N concentration were most obvious in SCH, where NO_3^--N concentration and exchangeable NH_4^+-N and TDN concentrations were twice as high in biocrusts, and in ALB, where the concentrations of TDN and DON decreased in biocrusts, even though none of them was significant (see Fig. 2 and Table 2). In SCH also, the amount of P_{H2O} was higher in biocrusts than in bulk soil. In contrast, P_{NaHCO3} was detected in lower concentrations in biocrusts of SCH and ALB.

Gene abundances

Absolute gene abundances were calculated as gene copy numbers g^{-1} dw and are displayed in Fig. 3, and the statistical results are shown in Tables 2 and 3. The differences between the values of each pair of biocrust and bulk soil are shown in Supplement Fig. 2.

Table 2 *p* values of linear mixed effect models. Significant values ($p < 0.05$) are shown in italics

	Variable	$p_{Exploratory}$	$p_{Sample\ type}$
Abiotic soil properties	N_{total}	*0.024*	0.283
	TDN	*0.011*	0.951
	NH_4-N	0.744	0.184
	NO_3-N	*0.042*	0.875
	DON	0.766	0.924
	P_{total}	*0.005*	0.707
	P_{NaHCO3}	0.081	0.143
	P_{H2O}	0.125	0.521
	N/P	0.284	0.265
	pH	*> 0.001*	0.322
Bacteria	16S rRNA gene	0.732	0.487
N cycle	*nifH*	*> 0.001*	0.344
	chiA	*> 0.001*	0.647
	apr	0.486	0.745
P cycle	*phoD*	*0.002*	*0.021*
	phnX	0.989	*0.007*
	gcd	0.298	*0.003*
	pstS	0.061	0.310
	pitA	0.894	*0.010*

Abundances were 1.1 (*pstS*, HAI) and up to 2.37 times (*pitA*, SCH) higher in biocrust samples for most of the genes. Regarding solubilization of P, the abundance of *gcd* gradually decreased from ALB to SCH from $2.3 \times 10^7 \pm 1.4 \times 10^7$ to $1.0 \times 10^7 \pm 8.3 \times 10^6$ mean gene copies g^{-1} dw. The abundance of *gcd* in SCH was the single significant difference between biocrust and bulk soil in P turnover, where *gcd* showed higher values in biocrusts. The abundances of the genes for the mineralization of P (*phoD*, *phnX*) were generally lowest. Especially, *phoD* was six to five times lower in SCH compared with ALB and HAI, respectively, and only reached $1.3 \times 10^6 \pm 6.1 \times 10^5$ copies g^{-1} dw in SCH. The gene abundance of *phnX* did not differ between regions. However, the *phnX* abundance in biocrusts of HAI was two times as high as in bulk soil of HAI. In SCH, the abundance of *phnX* was more than six times higher in biocrusts compared with the respective bulk soil. Regarding the abundance of marker genes for P uptake, *pstS* was higher compared with *pitA*, especially in ALB and HAI. The abundance of *pitA* in bulk soil and biocrusts was stable across all regions with mean gene abundances of $8.7 \times 10^6 \pm 5.1 \times 10^6$ and $1.5 \times 10^7 \pm 9.9 \times 10^6$ copies g^{-1} dw, respectively. In contrast, *pstS* abundance was lowest in SCH where at the same time the difference between biocrust and bulk soil ($r = 1.43$) was highest.

The gene abundances of microbes catalyzing N turnover were higher compared with genes triggering P turnover in all regions, except for *nifH* in SCH, which was in the same range as genes linked to P transformation processes. *nifH* was one order of magnitude higher abundant ($p < 0.015$) in ALB ($2.1 \times 10^8 \pm 1.6 \times 10^8$ copies g^{-1} dw) and HAI ($1.4 \times 10^8 \pm 9.2 \times 10^7$ copies g^{-1} dw) compared with SCH ($1.2 \times 10^7 \pm 9.4 \times 10^6$ copies g^{-1} dw). Moreover, the *nifH* gene abundance was nearly two times lower ($p = 0.029$) in the biocrust of the ALB compared with the bulk soil. The opposite trend was observed in SCH where in the biocrust, the *nifH* gene abundance was two times higher. The same pattern as for *nifH* was observed for *chiA* abundance with lowest values in SCH ($4.1 \times 10^7 \pm 1.8 \times 10^7$ copies g^{-1} dw) and highest in ALB ($2.1 \times 10^8 \pm 1.2 \times 10^8$ copies g^{-1} dw). Even though biocrusts and bulk soil did not differ significantly in *chiA* abundance, gene abundances in biocrusts of HAI and SCH were 1.3 and 1.5 times higher, respectively. The *apr* gene abundance did not differ between regions and sample type and ranged from 5.7×10^7 to 1.2×10^8 copies g^{-1} dw. Still, in SCH, 1.7 times more *apr* could be detected in the biocrust compared with the bulk soil. The abundance of bacterial 16S rRNA genes (see Fig. 3c) did not differ significantly between the regions or sample type (Table 2). Detected values varied from 3.4×10^{10} (bulk soil—SCH) to 5.96×10^{10} gene copies g^{-1} dw (biocrust—HAI).

Table 3 Significant p values of Tukey post hoc test of linear mixed effect models. Only variables being significantly affected by region or sample type are shown

	Variable	Contrast	$p_{\text{Post Hoc}}$
Abiotic soil properties	pH	ALB, bulk soil - SCH, bulk soil	> 0.001
		HAI, bulk soil - SCH, bulk soil	0.003
		ALB, biocrust - SCH, biocrust	> 0.001
		HAI, biocrust - SCH, biocrust	0.001
	N_{total}	ALB, bulk soil - SCH, bulk soil	0.039
	TDN	ALB, bulk soil - SCH, bulk soil	0.013
	P_{total}	ALB, bulk soil - SCH, bulk soil	0.016
		ALB, biocrust - SCH, biocrust	0.016
N cycle	*nifH*	ALB, bulk soil - SCH, bulk soil	> 0.001
		ALB, bulk soil - ALB, biocrust	0.029
		HAI, bulk soil - SCH, bulk soil	0.003
		ALB, biocrust - SCH, biocrust	0.011
		HAI, biocrust - SCH, biocrust	0.015
	chiA	ALB, bulk soil - SCH, bulk soil	0.002
		HAI, bulk soil - SCH, bulk soil	0.020
		ALB, biocrust - SCH, biocrust	0.022
		HAI, biocrust - SCH, biocrust	0.033
P cycle	*phoD*	ALB, bulk soil - SCH, bulk soil	0.005
		HAI, bulk soil - SCH, bulk soil	0.042
	gcd	SCH, bulk soil - SCH, biocrust	0.013

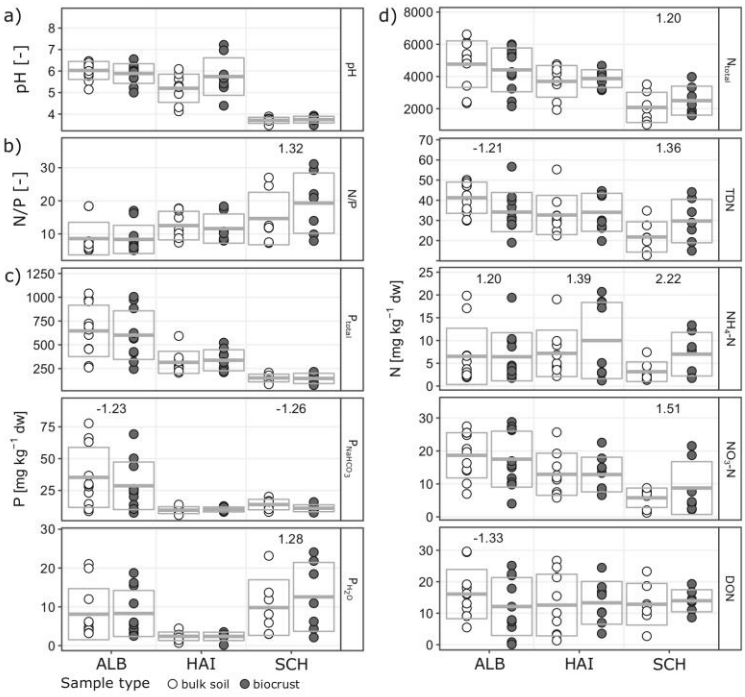

Fig. 2 Abiotic soil properties as pH (**a**), the N/P ratio (based on mg/kg) (**b**), the P (**c**), and N pools (**d**) are shown as dot plots and crossbars. Crossbars are marked by the mean values and standard deviation. The P pools include total P (P_{total}) and labile P (P_{H2O} and P_{NaHCO3}) and the N pools include total N (N_{total}), total dissolved N (TDN), exchangeable NH_4^+-N, NO_3^--N, and dissolved organic N (DON). The values are grouped by region and sample type. Above each group, the ratio r is displayed to see how biocrust and bulk soil differ on average. Values with $-1.1 \geq r \leq 1.1$ are not shown

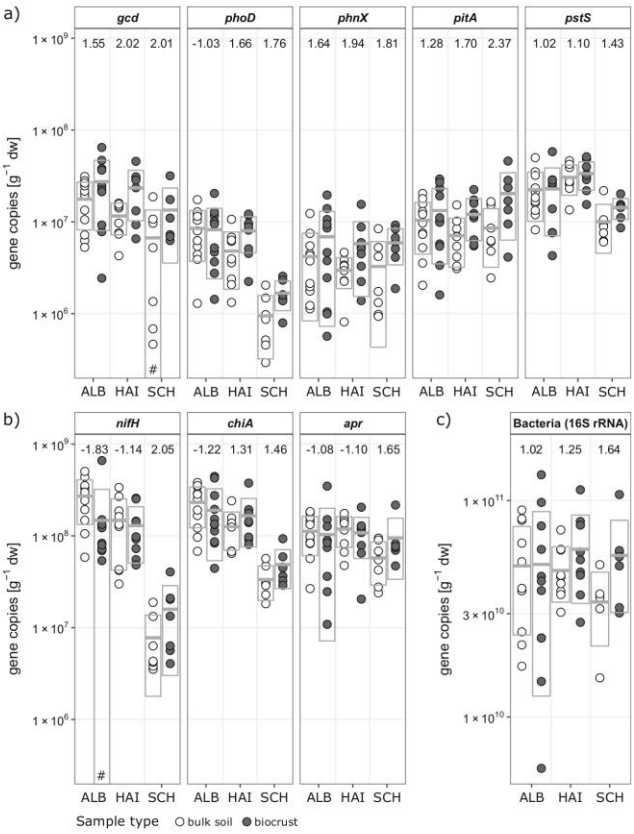

Fig. 3 Functional gene copy numbers per gram dry weight are shown as dot plots and crossbars. Crossbars are marked by the mean values and standard deviation and dots show the distribution of the single values. **a** P turnover (*gcd, phoD*, *phnX*, *pitA*, *pstS*), **b** N turnover (*nifH*, *chiA*, *apr*), and **c** the abundance of the 16S rRNA gene as marker for total bacterial abundance. "#" indicates that standard deviation is higher than the mean value. The values are grouped by region and sample type. Above each group, the ratio *r* is displayed to see how biocrust and bulk soil differ on average

To also account for minor changes in bacterial biomass between bulk soil and biocrust, relative gene abundances were calculated as copy numbers per 16S rRNA gene abundance. The results are shown in Supplement Fig. 3 and statistical results are shown in Supplement Table 2 and 3. Regarding N cycle genes, tendencies of relative gene abundances were similar to absolute gene abundances, and only for *nifH* gene abundances, differences between bulk soil and biocrust in ALB were less pronounced. Also, for P cycle genes, the pattern of relative gene abundances was mostly comparable with those of the absolute gene abundances with more pronounced differences between exploratories for *phoD* relative gene abundance and between bulk soil and biocrust in HAI for *pitA* relative gene abundance. Only, the relative gene abundance of *gcd* was higher in the bulk soil of SCH, while it was higher in the biocrust for the absolute gene abundances.

Correlation of soil chemical properties and gene abundances

In total, 18 variables were included in the correlation analyses. The significant Pearson correlations ($r^2 > 0.6$, $r^2 < -0.6$, $p < 0.05$) are shown in Fig. 4. From Table 4 and Fig. 4, it can be concluded that the total number of significant correlations is comparable between sample types in each region but the type of connections can be quite different. In general, the total number of correlations decreased from ALB and SCH to HAI, while the number of negative correlation was lowest in ALB and highest in SCH.

In particular, in ALB, the difference in numbers of correlations was highest between biocrust and bulk soil and resulted in 30 and 42 positive correlations, respectively. Here, we found strong correlations of genes driving N and P turnover with each other. The P pools revealed a strong intra-correlation in bulk soil and biocrusts in ALB. In bulk soil,

Table 4 Number of significant Pearson correlations as shown in Fig. 4

	ALB		HAI		SCH	
	Bulk soil	Biocrust	Bulk soil	Biocrust	Bulk soil	Biocrust
$r^2 > 0.6$	42	30	29	26	27	29
$r^2 < -0.6$	1	5	7	4	9	12
Total sum per sample type	43	35	30	36	36	41

TDN and NO_3^--N concentrations were correlated with all abundances of genes, except *phnX* to TDN. In contrast, TDN concentration was only correlated to *phoD* abundance and NO_3^--N concentrations to *chiA* abundance in biocrusts while the other abundances of genes (*pstS*, *phnX*, *nifH*, and *apr*) were correlated to exchangeable NH_4^+-N concentration. Interestingly, no correlations for *nifH* abundances were found in bulk soil, even though it was the highest abundant gene in bulk soil of ALB compared with all other samples. However, we detected a strong correlation between *nifH* abundance and exchangeable NH_4^+-N concentrations in biocrusts.

In HAI, we observed fewest correlations compared with the other regions ($n_{total} = 66$). In bulk soil, a strong correlation of *phoD*, *pitA*, and *chiA* abundances could be observed, while *pstS* abundance was not correlated even though it was highly abundant. pH was strongly correlated in biocrust and bulk soil, in biocrusts especially to abundances of P turnover genes. In biocrusts, a similar correlation pattern between gene abundances was found as described for ALB.

The highest number of negative correlations was detected in SCH ($n_{total} = 77$, $n_{r^2<0} = 21$) which were equally distributed between biocrust ($n = 29$, $n_{r^2<0} = 12$) and bulk soil ($n = 27$, $n_{r^2<0} = 9$). In bulk soil, *chiA* and *nifH* abundances revealed the highest number of correlations, mostly with abundances of genes involved in P turnover. We found a strong correlation of *pitA* and *nifH* abundances, and N_{total}, which was also obvious in bulk soil of HAI, but weaker. Further, P_{NaHCO3} concentration was negatively correlated to pH, *phoD*, *chiA*, and *apr* abundances in bulk soil and to TDN and exchangeable NH_4^+-N concentrations in biocrusts of SCH samples. No correlations for *pstS* abundance in biocrusts were found, even though it was much higher abundant compared with bulk soil.

Discussion

Parent material determines P acquisition strategy in biocrusts

The aim of this study was to investigate the correlation of the bacterial driven P and N turnover in biocrusts from forests, which differ in nutrient stocks, soil texture, and pH. To be able to correlate nutrient concentrations and abundance of major functional microbial groups, we did one extensive sampling campaign during peak biomass of the biocrusts. In nutrient-poor habitats, biocrusts are important first colonizers and described as "mantels of fertility" by Garcia-Pichel et al. (2003) as they are known to increase nutrient concentrations due to activities of C and N_2 fixing phototrophic organisms and thus contain higher abundances of microorganisms compared with bulk soils in arid environments (Castillo-Monroy et al. 2011; Maier et al. 2018). In the forest biocrusts, we neither detected significantly higher nutrient concentrations nor significantly increased bacterial abundance (16S rRNA gene) in most of the biocrusts (Table 2), even though well-developed biocrusts were present at all investigated plots (see Fig. 1) which was further corroborated by the conspicuous presence of algae (Glaser et al. 2018). The well-developed forest soils are generally rich in nutrients and organic substances, which allow for already high microbial abundances in bulk soil. For example, the sample with the lowest bacterial abundance reached 3.4×10^{10} gene copies g^{-1} dw (bulk soil—SCH), which can be hardly reached in biocrusts from arid environments but is definitely several orders of magnitude higher compared with the respective bulk soils (e.g., Couradeau et al. 2016; Maier et al. 2018; Nagy et al. 2005). Moreover, in temperate forests, biocrusts are mostly only temporally abundant during spring, when soil temperature is above freezing point and light is available in sufficient photon flux rates before the vegetation period. Further, strong disturbances of biocrusts, e.g., by management, are likely. Both factors result in high dynamics of biocrust development in temperate forests and yearly formations are typical for such environments. Only at the early vegetation period, the additional input of C by the phototrophic organisms may favor the establishment of a biocrust community. The combination of high nutrient concentrations in bulk soil and short periods of biocrust formation and persistence explain the less pronounced difference of bacterial abundance and nutrient concentrations between biocrusts and bulk soil.

In ALB, the P pools and gene abundances of bacteria involved in P turnover did not differ significantly between bulk soil and biocrust (Table 3). In contrast in HAI and SCH, the absolute and relative abundance of bacteria specifically involved in P turnover increased much more in the biocrust, while P_{total} did not differ in any region. P_{NaHCO3} on the other hand was slightly decreased in biocrusts compared with bulk

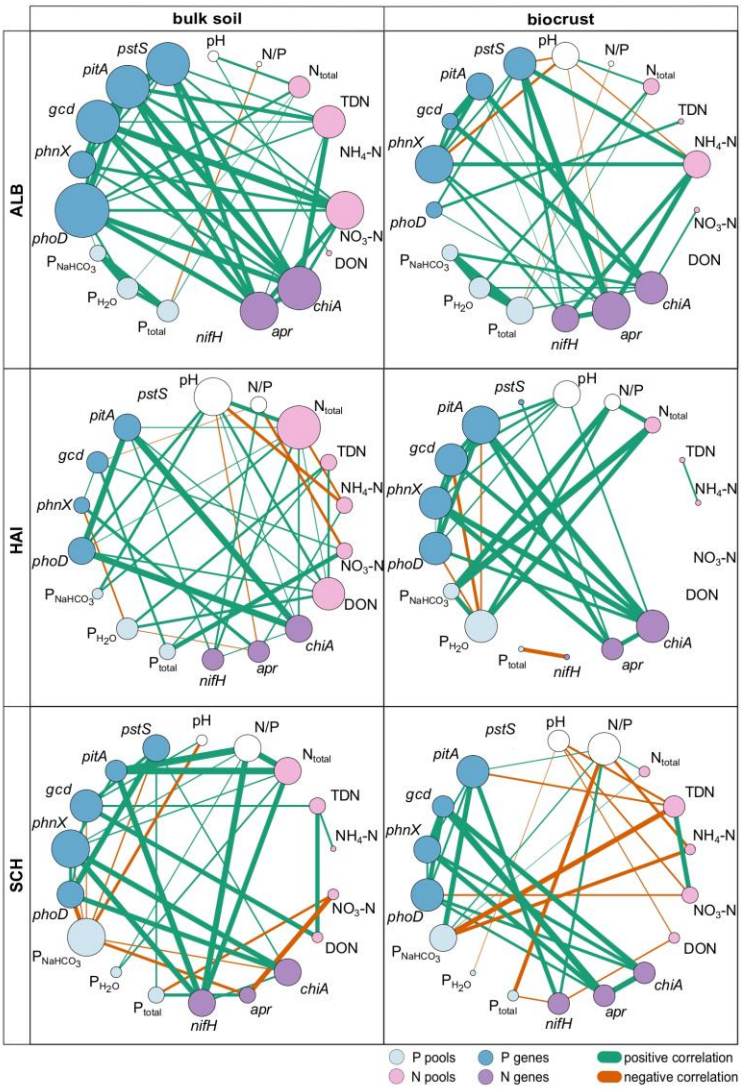

Fig. 4 Correlations within biocrusts and bulk soil are shown as Pearson correlation plot for each region. The analysis included pH, N/P ratio (based on mg/kg), N pools (N_{total}, TDN, NH_4^+-N, NO_3^--N, DON), P pools (P_{total}, P_{H2O}, P_{NaHCO3}), as well as the gene abundance for N (*nifH*, *chiA*, *apr*) and P cycle genes (*phoD*, *phnX*, *gcd*, *pitA*, *pstS*); those categories are indicated by circle colors. The size of each circle indicates the degree of correlations while the thickness of each line indicates the strength of correlation, ranging between $r^2 = 0.6$ and $r^2 = 0.97$ with $p < 0.05$

soil in ALB and SCH. Moreover, the ALB had highest *gcd* gene abundances, indicating the highest potential for P solubilization. Solubilization was also identified as key process in other P-rich forest regions (Bergkemper et al. 2016a) as well as in agricultural fields with sufficient P supply (Grafe et al. 2018). The additionally high P_{NaHCO3} concentration might point to an efficient release of P from iron and aluminum oxides, which have a good solubility in the pH range of the ALB (Lindsay 1979).

In comparison with ALB, the P_{total} concentrations of HAI and SCH were significantly lower. Interestingly, the bacterial potentials to acquire P in the biocrusts differed between those two sites with respect to P uptake and mineralization of organic P. In HAI, similar to ALB, the mineralization of P in biocrusts was dominated by bacteria carrying the *phoD* gene, which are able to hydrolyze phosphomonoesters and –diesters under favorable conditions. Furthermore, the labile P pools were very low in HAI, which coincides with the highest

abundance of the phosphate-inducible transporter gene *pstS*. In biocrusts and bulk soil from SCH, bacteria carrying the *phnX* gene were much more abundant than *phoD* and increased in biocrusts. However, as our analysis was done on DNA level, we cannot conclude that this is also true for the expression of those genes. For P-poor soils, it was demonstrated that the pool of easily degradable phosphomono- and diesters is depleted quickly, and that instead more recalcitrant phosphonates remain in the soil as organic P source (Bergkemper et al. 2016a; Condron et al. 2005; Lang et al. 2017). For SCH, this could indicate that the supply with mono- or diesters is not high enough, so that the P demand is fed by the additional degradation of phosphonates. In this regard, Baumann et al. (2017) concluded that P solubilized from the minerals is directly transferred and immobilized by the microbiome of the biocrusts, because the combination of low pH and sandy texture reduces the sorption capacity of soils in SCH, as low concentrations of iron and aluminum minerals can be assumed (Lang et al. 2017). This is further underlined by the high P_{H2O} and low P_{NaHCO3} concentrations. Thus, immobilization by sorption to minerals is unlikely and corroborated by the significantly reduced relative abundance of *gcd* in biocrusts of SCH, even though absolute *gcd* numbers slightly increased in biocrusts their relative abundance compared with total 16S rRNA gene abundance dropped. Additionally, an increase of *pstS* was observed in the biocrusts of SCH. *pstS* is part of the *Pho* regulon and important for P uptake under P limitation (Santos-Beneit 2015). Consequently, during biocrust development, P might get limited and uptake via the *pstS* pathway displays an advantage. Surprisingly, also the abundance of *pitA* was higher in biocrusts from SCH compared with the other sites. The *pitA* gene codes for the unspecific P_i transporter (Wanner 1993; Willsky and Malamy 1980). Thus, bacteria from these biocrusts are well equipped for the uptake of P under fluctuating conditions.

Labile pools determine the correlation of bacterial P and N turnover

For forests, Cleveland and Liptzin (2007) postulated a well-constrained molar N/P ratio of 9:1 for microbial biomass and of 15:1 for forest soils. Regarding the different sites of our study, a similar ratio between N and P in the soil were measured for the ALB (N/P ratio of 8.5 ± 4.6), while the difference between N_{total} and P_{total} concentrations increased from HAI to SCH. Thus, we assumed that in ALB processes to acquire N and P are closely interlinked, while in HAI and SCH, the microbes are facing P limitation which is in line with higher potentials for P acquisition.

Regarding ALB, our data underlines a strong link between N and P turnover; both abundances of N mineralization genes (*apr*, *chiA*) measured are significantly correlated to abundances of all P cycle genes. However, it has to be taken into account that there are much more enzymes able to mineralize organic N sources, like neutral proteases or subtilisin, than the ones which we analyzed in this study. Nonetheless, our chosen marker genes cover chitinolytic and proteolytic proteins. Moreover, our observation is based on statistical analyses, with a lack of additional experimental testing, for example, by fertilization, which is a classical drawback of field studies, but compensated by a high number of replicates in this study (up to 11 replicates). The strong correlation between N and P cycle genes might indicate that the mineralization of organic sources is used to deliver both N and P to the same extent. A similar observation was described by Heuck and Spohn (2016), who demonstrated in an incubation experiment of organic horizons from temperate forests that net N and P mineralization are strongly correlated and increased with decreasing N/P ratio. Against this backdrop, it is not surprising that the abundance of bacteria harboring the *nifH* gene was generally highest in the ALB and contrary to our hypothesis, significantly increased in the bulk soil compared with the biocrust ($p = 0.029$). Taken into account that the fixation of one molecule N_2 needs 16 molecules of ATP (Zehr et al. 2003), a relatively better supply with P and also C could potentially increase N_2 fixation rates (Reed et al. 2007). Thus, in the ALB, this displays an additional source to fuel the labile N pool. Even though the *nifH* gene abundance was significantly lower in the biocrusts, the nutrient pools did not differ between bulk soil and biocrusts in the ALB, which is not compensated by higher N mineralization gene abundance. However, we also have to consider that *nifH* is phylogenetically widely distributed (Gaby and Buckley 2012) and that the primers used only cover sequences of *nifH* cluster I, which comprises aerobic N fixers like *Proteobacteria*, *Frankia*, *Paenibacillus*, and some *Cyanobacteria* like *Anabaena*, *Nostoc*, and *Plectonema* but excludes others, which might be associated with biocrusts (Rösch et al. 2002). Within this study, we also determined the nitrification potentials as revealed by the quantification of the *amoA* marker gene coding for a subunit of the ammonia monooxygenase of bacteria and archaea. And indeed, when *nifH* was increased in bulk soils, also ammonia-oxidizing bacteria or archaea were increased in over 70% of the replicates (data not shown). The ammonium produced during N_2 fixation might be used as substrate for the nitrification process, which in turn might have caused a reduction of exchangeable NH_4^+-N. This hypothesis is supported by a strong positive correlation of *nifH* abundance to NH_4^+-N in the biocrusts, which is a plausible hint that the NH_4^+-N is not immediately oxidized to NO_3^--N in the biocrusts. Moreover, Sorkau et al. (2018) demonstrated that 86% of microbial P variation in the forest soils of the same regions can be explained by microbial N while also organic C, soil moisture, and soil type were main drivers for microbial P. A limitation of P is unlikely in ALB, neither in biocrusts nor in

bulk soil, as we could detect high P_{total}, quite high P_{NaHCO3} concentrations, and high potentials for solubilization (*gcd*).

Only in SCH, the abundance of the microbes which drive N and P turnover was always higher in the biocrust compared with the bulk soil samples. Moreover, their abundances were always positively correlated with each other. Probably, the sandy soil and the generally low nutrient stocks in SCH rather reflect a nutrient-poor habitat, where biocrusts are described as nutrient and microbial hotspots (Brankatschk et al. 2013; Hernandez and Knudsen 2012; Kidron et al. 2015; Schulz et al. 2013; Xiao and Veste 2017). While total nutrient stocks were significantly lowest in SCH, the labile nutrient pools were comparable with the other regions or even higher in the biocrusts for P_{H2O}. One explanation might be that the P and N mineralization and subsequent accumulation were driven by the microbial need for C. This phenomenon was already described in other forest ecosystems (Spohn and Kuzyakov 2013) but also in glacier forefields where especially at well-developed sites, microbes were limited by C rather than N or P (Göransson et al. 2011). Consequently, N and P remain in the soil and built up a higher labile nutrient pool. Moreover, the transcription of the ammonium transporter gene *amtB* is under the control of the *Pho* regulon (Santos-Beneit 2015). Thus, under P starvation, also NH_4^+-N uptake is reduced.

In contrast to SCH and ALB, lowest number of correlations were found in HAI as well as lowest labile P concentrations, while exchangeable NH_4^+-N was highest in HAI and even enriched in the biocrusts but less than the ones from SCH. On the one hand, this could point towards different uptake strategies for N and P or on the other hand different mineralization activities. However, as this study is based on DNA analyses, which only reflect the bacterial potential and nutrient pools in the soil, it is impossible to disentangle these strategies. Biocrusts are small micro-habitats, which do not allow to sample large amounts, which is for example needed for additional microbial biomass analyses or RNA sampling, as therefore much more sub-samples per biocrusts need to be analyzed to account for daily fluctuations of gene transcription pattern. However, what was remarkable in bulk soil samples from HAI was the prominent correlation of *pitA*, *chiA*, and *phoD* abundance. This would rather point towards a specific mineralization of either N- or P-containing sources in HAI bulk soil samples, as chitin for example does not contain any P moieties. What was conspicuous in HAI was the high variability of the pH, which ranged from pH 4.13 to 7.22. This might mask a lot of effects, as pH differences are frequently described as main driver for differences in other soil properties, microbial community composition, and functionality (Fierer and Jackson 2006; Rousk et al. 2010; Stempfhuber et al. 2015, 2017). In the frame of the Biodiversity Exploratories, Stempfhuber et al. (2017) demonstrated that pH has a strong impact on the nitrifying community as the ratio of ammonium to ammonia is changed and causes changes in potential activities and the community composition. The lower the pH, the more ammonia-oxidizing archaea prevail, which have a lower turnover rate (Stempfhuber et al. 2017). Thus, especially the extremely low pH values measured in the HAI biocrusts might have caused an accumulation of exchangeable NH_4^+-N.

Conclusion

In conclusion, our data demonstrate that the potential to biogeochemically turnover P is an essential microbial function in biocrusts, as such microbes were more abundant in the biocrusts compared with bulk soil at all investigated regions on the level of absolute and relative abundance. In contrast to our initial hypothesis that low nutrient concentrations cause a strong correlation of N and P turnover, we found most positive correlations in the region with the highest nutrient concentrations but the lowest N/P ratio. Especially, the potential for additional N input by fixation seems to be of importance at sites with low N/P ratios. Potential N mineralization was strongly correlated to P turnover at sites where labile N and P pools were increased. Even though the correlation of N and P turnover is strongest if their availability is comparable, the potential acquisition strategy obviously strongly depends on soil properties. Future studies should confirm the abundance pattern of certain functional microbial groups in subsequent years. However, as biocrusts in temperate regions newly develop every year at distinct places, a direct comparison is often difficult, as (micro)environmental conditions may differ even if the same site is analyzed. Additionally, despite the short life time of biocrusts mainly in temperate forests, also repeated samplings during the succession would be of interest to understand tipping points of different developmental stages. This would need possibilities for a miniaturization of analytical tools to ensure the availability of material for all needed analysis as a result of the small sizes of biocrusts. However, especially, in regions with inconclusive results as in HAI, in-depth microbial community analyses including non-targeted metagenomic approaches might help to disentangle region-specific and biocrust-specific effects. As our data based on measurements on DNA level future work should consider combining microbial activity measurements and actual turnover rates to identify under which conditions the detected potential is accessed.

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Data availability All data generated or analyzed during this study are included in this published article and its supplementary information files. Additionally, datasets are available in the BExIS database of the Biodiversity Exploratories project: ID 26166 (https://www.bexis.uni-jena.de/PublicData/PublicDataSet.aspx?DatasetId=26166) and ID 26206 (https://www.bexis.uni-jena.de/PublicData/PublicDataSet.aspx?DatasetId=26206).

Compliance with ethical standards

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

Ethics approval Not applicable.

Consent to participate The authors declare that they consent to participate.

Consent for publication The authors declare that they consent for publication.

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Supplementary Material

Correlation of the abundance of bacteria catalyzing phosphorus and nitrogen turnover in biological soil crusts of temperate forests of Germany

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Supplement Table 1 Number of biocrusts sampled per exploratory and plot

Exploratory	Plot	$n_{biocrust}$
ALB	AEW 5	2
	AEW 18	3
	AEW 40	3
	AEW 43	3
HAI	HEW 6	3
	HEW 21	3
	HEW 47	3
SCH	SEW 5	1
	SEW 35	1
	SEW 38	3
	SEW 49	2

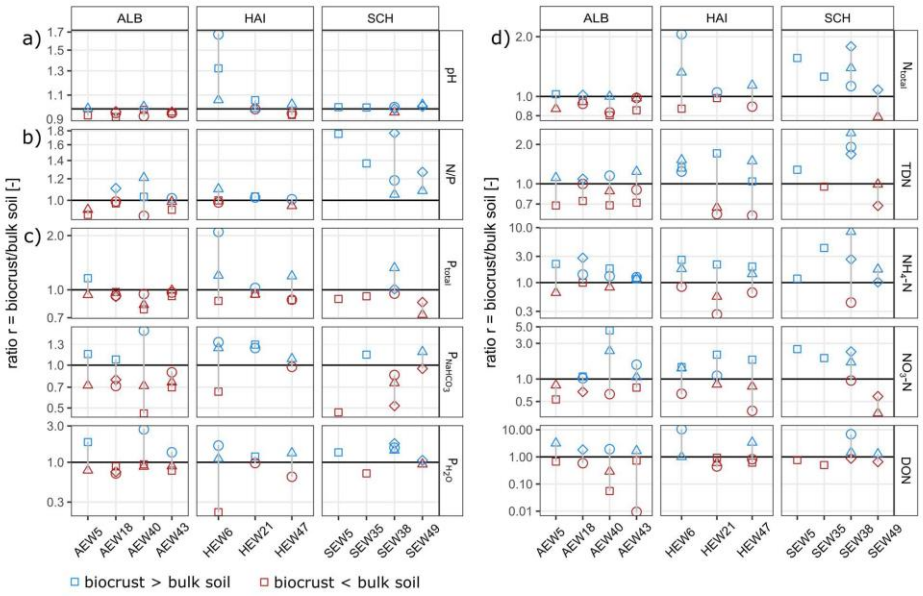
Supplement Table 2 p values of Linear Mixed Effect Models for relative gene copy numbers per 16S rRNA gene copy number. Significant values ($p<0.05$) are shown in bold.

	Variable	$p_{Exploratory}$	$p_{Sample\ type}$
N cycle	*nifH*	**> 0.001**	**0.044**
	chiA	**> 0.001**	0.340
	apr	0.371	**0.049**
P cycle	*phoD*	**> 0.001**	**0.012**
	phnX	0.523	**0.004**
	gcd	0.372	**0.011**
	pstS	**0.049**	0.885
	pitA	0.111	**> 0.001**

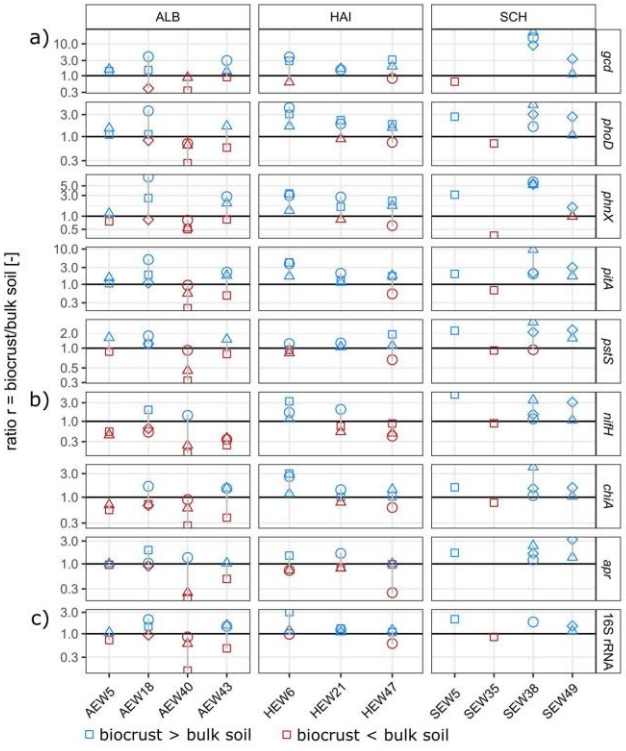
Supplement Table 3 Significant p values of Tukey Post Hoc Test of Linear Mixed Effect Models for relative gene copy numbers per 16S rRNA gene copy number. Only variables being significantly affected by region or sample type are shown.

	Variable	contrast	$p_{Post\ Hoc}$
N cycle	*nifH*	ALB, bulk soil - SCH, bulk soil	> 0.001
		HAI, bulk soil - SCH, bulk soil	0.010
		ALB, biocrust - SCH, biocrust	0.007
		HAI, biocrust - SCH, biocrust	0.025
	chiA	ALB, bulk soil - SCH, bulk soil	> 0.001
		HAI, bulk soil - SCH, bulk soil	0.025
		ALB, biocrust - SCH, biocrust	> 0.001
		HAI, biocrust - SCH, biocrust	0.005
P cycle	*phoD*	ALB, bulk soil - SCH, bulk soil	0.003
		HAI, biocrust - SCH, biocrust	0.012
		ALB, biocrust – SCH, biocrust	0.002
	pitA	HAI, bulk soil – HAI, biocrust	0.047

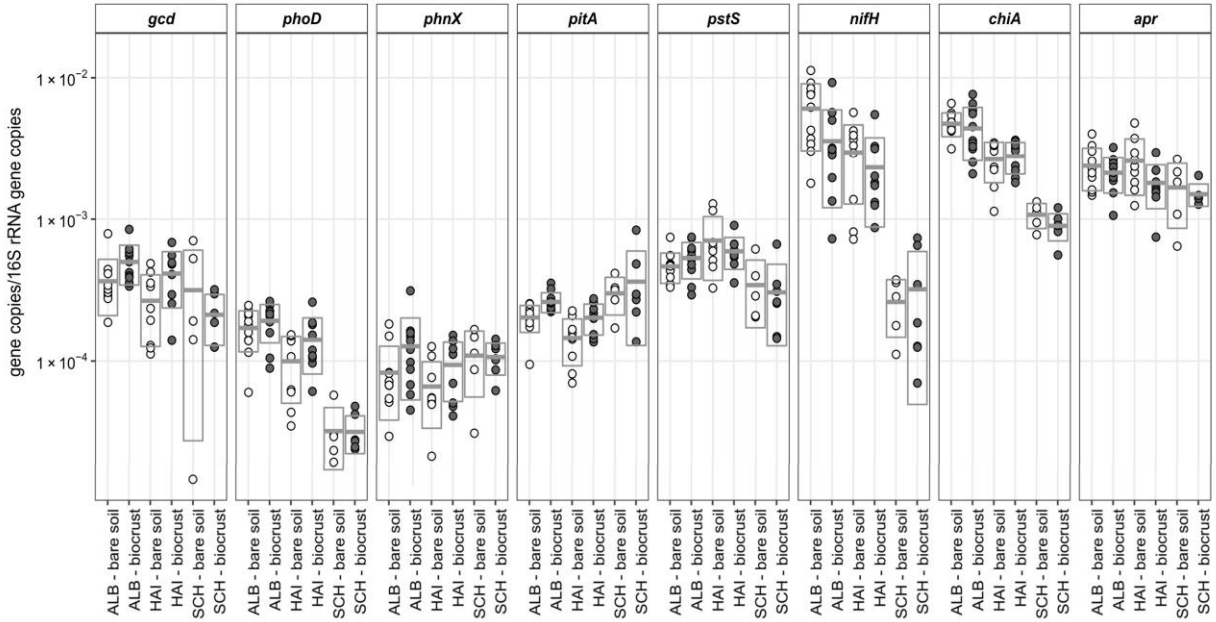
Supplement Figure 1 Ratio for abiotic soil properties on logarithmic scale (log10) as pH (a), the N:P ratio (based on mg/kg) (b), the phosphorous (c) and the nitrogen pools (d) as shown in dotplots for every replicate (marked by shape) per plot. The phosphorus pools include total phosphorus (P_{total}) and labile phosphorus (P_{H2O} and P_{NaHCO3}) and the nitrogen pools include total nitrogen (N_{total}), total dissolved nitrogen (TDN), ammonium (NH_4-N), nitrate (NO_3-N) and dissolved organic nitrogen (DON). The black line indicates r = 1 where biocrust and bulk soil values are equal. Blue signs show where biocrust values where higher than bulk soil and red signs show where bulk soil values where higher than biocrusts.



Supplement Figure 2 Ratio for functional gene copy numbers per gram dry weight are shown as dotplots for every replicate (marked by shape) per plot on logarithmic scale (log10) for a) P turnover (*phoD, phnX, gcd, pitA, pstS*), b) N turnover *(nifH, chiA, apr*), and c) the abundance of the 16S rRNA gene as marker for total bacterial abundance. The black line indicates r = 1 where biocrust and bulk soil values are equal. Blue signs show where biocrust values where higher than bulk soil and red signs show where bulk soil values where higher than biocrusts.



Supplement Figure 3 Relative abundance of functional genes**.** Functional gene copy numbers per gram of soil were divided by 16S rRNA gene copy numbers per gram of soil and are shown as dotplots and crossbars. Crossbars are marked by the mean values and standard deviation and dots show the distribution of the single values. P turnover is displayed by *gcd*, *phoD*, *phnX*, *pitA*, *pstS* and N turnover by *nifH*, *chiA* and *apr*.



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Biological soil crusts on agricultural soils of mesic regions promote microbial cross-kingdom co-occurrences and nutrient retention

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Introduction: Biological soil crusts (biocrusts) are known as biological hotspots on undisturbed, nutrient-poor bare soil surfaces and until now, are mostly observed in (semi-) arid regions but are currently poorly understood in agricultural systems. This is a crucial knowledge gap because managed sites of mesic regions can quickly cover large areas. Thus, we addressed the questions (i) if biocrusts from agricultural sites of mesic regions also increase nutrients and microbial biomass as their (semi-) arid counterparts, and (ii) how microbial community assemblage in those biocrusts is influenced by disturbances like different fertilization and tillage regimes.

Methods: We compared phototrophic biomass, nutrient concentrations as well as the abundance, diversity and co-occurrence of Archaea, Bacteria, and Fungi in biocrusts and bare soils at a site with low agricultural soil quality.

Results and Discussion: Biocrusts built up significant quantities of phototrophic and microbial biomass and stored more nutrients compared to bare soils independent of the fertilizer applied and the tillage management. Surprisingly, particularly low abundant Actinobacteria were highly connected in the networks of biocrusts. In contrast, Cyanobacteria were rarely connected, which indicates reduced importance within the microbial community of the biocrusts. However, in bare soil networks, Cyanobacteria were the most connected bacterial group and, hence, might play a role in early biocrust formation due to their ability to, e.g., fix nitrogen and thus induce hotspot-like properties. The microbial community composition differed and network complexity was reduced by conventional tillage. Mineral and organic fertilizers led to networks that are more complex with a higher percentage of positive correlations favoring microbe-microbe interactions. Our study demonstrates that biocrusts represent a microbial hotspot on soil surfaces under agricultural use, which may have important implications for sustainable management of such soils in the future.

KEYWORDS

biocrust, amplicon, hotspot, managed site, Cyanobacteria, microbial co-occurrence network, mesophilic region

1. Introduction

Biological soil crusts (biocrusts) are an assemblage of soil particles, photoautotrophic primary producers, heterotrophic microorganisms and microfauna on or within the first few millimeters of the soil surface (Weber et al., 2022). They are particularly abundant in arid and nutrient-poor environments (Belnap, 2003; Maier et al., 2018). In those ecosystems biocrusts fulfill important functions related to carbon and nitrogen fixation, the storage of water and nutrients as well as the induction of soil formation and stabilization (Belnap et al., 2001; Costa et al., 2018). Phototrophic biota such as Cyanobacteria, microalgae, or mosses are essential key-players to provide such functions (Miralles et al., 2012). For example, Cyanobacteria can stabilize the soil matrix due to their filamentous growth (Chamizo et al., 2018; Jung et al., 2018) and the production of sticky exopolysaccharides (Cania et al., 2019a). The fixation of carbon and nitrogen attracts heterotrophic microbes including Bacteria, Fungi, Archaea and Protists (Baumann et al., 2018; Maier et al., 2018; Roshan et al., 2021). As a consequence, Cyanobacteria are often described as keystone taxa of biocrusts in studies, which investigated microbial community composition and performed correlation network analyses (Chilton et al., 2018; Pombubpa et al., 2020).

There is increasing evidence that biocrust formation is not confined to nutrient-poor and arid regions. Recent studies identified biocrusts in managed ecosystems of mesic regions (Gall et al., 2022) like forests (Baumann et al., 2017; Glaser et al., 2018; Kurth et al., 2021; Glaser et al., 2022a). In contrast to arid biocrusts, they occur here as ephemeral stages. In forests, it was demonstrated that the biomass of biocrusts quickly increased in spring before herbal plant growth or after disturbance events like tree cutting or wind driven tree fall, which gives phototrophic biota of biocrusts a selective advantage (Kurth et al., 2021). Similar to forests, agroecosystems also provide potential niches for biocrust development, such as the time between harvest and sowing or between the rows of broad leave crops like corn, potatoes, or sugar beets. Besides the potential time and space for biocrust development in agroecosystems, multiple studies have reported that single components of agricultural management including tillage (Curaqueo et al., 2010; Wang et al., 2016; Wagg et al., 2018) and fertilization are detrimental for microbial community assembly, which might hamper the development of biocrust communities (Brankatschk et al., 2013; Schulz et al., 2013; Maier et al., 2018). Additionally, the previously described central role of Cyanobacteria in microbial networks might be obsolete in biocrusts of fertilized soils, because of the high external nutrient input. However, 'on-farm' studies, which investigate the combined influence of tillage intensity and fertilizer type or amount on microbial community composition, are still missing. Taking the positive effect of biocrusts on many ecosystem functions and their potential importance for sustainable agricultural management into account, there is a strong need to overcome this limitation.

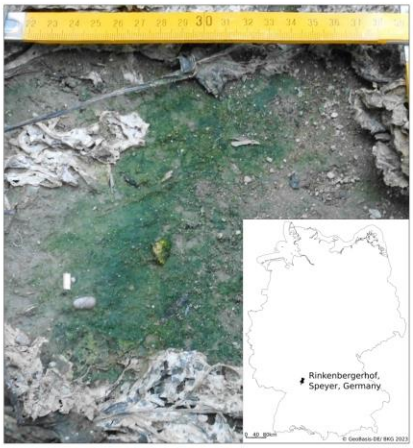
Thus, this study aimed to investigate the combined effect of mineral fertilization and organic treatments as well as tillage intensity on: (1) the composition and correlation of microbial communities in biocrusts of an agricultural field and (2) the ability of those biocrusts to further increase the amount of available nutrients. Samples were taken under the auspices of a long-term fertilization experiment in Germany, which combined different tillage intensities (minimal, reduced, conventional tillage) with different fertilization treatments (different levels of mineral nitrogen fertilizer, with or without crop residue retention). Long-term data revealed already that reduced tillage and crop residue retention increased carbon stocks and sugar beet yields in this experiment (Armbruster et al., 2009, 2012). Sampling was conducted in autumn at the end of sugar beet cultivation but before harvest, which allowed a maximum period for biocrust development. Samples were taken between sugar beet rows. We analyzed nutrient concentrations, the community composition of Archaea, Bacteria and Fungi as well as their co-occurrence and mutual exclusion pattern in biocrusts in comparison to the surrounding bare soil.

2. Methods

2.1. Experimental field and sampling procedure of biocrusts

The current study was conducted 2016 at the agricultural experimental farm "Rinkenbergerhof" in Germany (49°21′34.0"N 8°25′14.8"E), which belongs to the Agricultural Investigation and Research Institute Speyer (Figure 1). Samples were taken in frame of the long-term "International Organic Nitrogen Fertilization Experiment (IOSDV)," which is carried out since 1983. The site is characterized by a mean annual temperature of 10.0°C and a mean annual precipitation of 593 mm. The soil of the IOSDV experiment has been described as Cambisol consisting of equal amounts of silt and sand and 9% of clay. It has a field capacity of 10%. The German arable assessment ("Ackerzahl") evaluated the soil with 25 to 30, which represents sites with a low soil quality (Bischoff and Emmerling, 1997; Armbruster et al., 2012; Schmid et al., 2018). Phosphorus, potassium and magnesium were applied in equal amounts to all plots (31 kg of phosphorus ha^{-1} $yr.^{-1}$, 121 kg of potassium ha^{-1} $yr.^{-1}$ and 28 kg of magnesium ha^{-1} $yr.^{-1}$). In 2016 during sugar beet cultivation pesticides including fungicides were used based on the particular needs and irrigation of 110 mm was applied to avoid drought damage of the sugar beet plants.

The IOSDV experiment investigates the combination of mineral fertilization and organic treatments (since 1983) as well as tillage intensity (since 2004) in a 3 years crop rotation of sugar beet, winter wheat, and winter barley (Bischoff and Emmerling, 1997; Armbruster et al., 2012; Schmid et al., 2018). The experiment is designed in a full-factorial block design with three replicates per treatment and a plot size per treatment of 6 m * 7.5 m. In frame of the experiment, we sampled the following treatments: (1) 120 and 240 kg N ha^{-1} $yr.^{-1}$ mineral nitrogen fertilization (calcium ammonium nitrate). The applied rates are typical rates used in low-and high input agriculture in the region of the study, respectively. (2) We compared the additional organic treatment (+org) to control plots (−org). This was characterized by the retention of crop residues after harvest and the cultivation of a cover crop (*Raphanus sativus* var. *oleiformisis*) every third year after winter barley cultivation. (3) Tillage intensity was investigated by sampling plots with reduced (rT) and conventional tillage (cT). During rT the soil is broken up by a cultivator to a depth of 10 cm without turning the soil and under cT, the soil is plowed to 30 cm. Because of the different treatments, carbon stocks varied between the treatments.



Mineral Fertilizer Amounts	Organic treatment	Tillage	
		conventional	reduced
120kg N ha^{-1} yr^{-1}	without crop residues	cT 120 -org cT 120 +org	rT 120 -org rT 120 +org
240kg N ha^{-1} yr^{-1}	without crop residues	cT 240 -org cT 240 +org	rT 240 -org rT 240 +org

FIGURE 1
Picture of a biocrusts from a plot with conventional tillage, without crop residues and a mineral fertilization amount of 240 kg N ha^{-1} $yr.^{-1}$, taken on October 5th, 2016. Integrated is a map of Germany showing the location of the experimental field. The table below the figure shows the details of treatment combinations for tillage, mineral fertilizer and organic treatment. Tillage management is compared between conventional (cT) and reduced tillage (rT). The effect of mineral fertilizer amounts is evaluated in levels of 120 and 240 kg N ha^{-1} $yr.^{-1}$. Organic treatment as crop residues (+org) are compared to a control plot (−org) where crop residues were removed after harvesting.

Sampling took place on October 5 in 2016. The sampling time was chosen to allow for a maximal development of biocrusts and prior to the harvest of sugar beets, which would destroy the biocrusts, because of the heavy machinery used. On some plots, the biocrusts were so prevalent, that reference sampling of bare soil was challenging, which might have been caused by high precipitation of 25 mm in the 2 weeks before sampling. However, during the sampling and right before there was no rain. In total, 24 biocrusts and 24 bare soil samples were taken, which consisted of three replicates of the eight treatment combinations: cT 120 −org, cT 120 +org, cT 240 −org, cT 240 +org, rT 120 −org, rT 120 +org, rT 240 −org, rT 240 +org (see Figure 1). Biocrusts were visually identified as green covered soils according to the biocrust definition of Weber et al. (2022). Biocrusts were sampled from the top millimeters of the mineral soil between the sugar beet rows and were lifted with a spatula and sampled as a coherent layer of approximately 3 mm thickness. Most of the biocrusts were located in tractor traffic lanes and in little grooves (Supplementary Figure S1). Green biofilms on macroscopic organic matter like decomposed leaves were not considered as biocrusts. Samples from biocrust-free areas (no visible phototrophic biomass), referred to as bare soil, were taken from the top 3 millimeters. A composite of five biocrust and bare soil samples per plot was homogenized to reach sufficient amounts of sampling material. All samples were sieved to a particle size of 2 mm. Approximately, 12 g of fresh soil was stored at 4°C for chemical analysis and *ca.* 2 g was shock frozen on dry ice in the field and stored at −80°C for subsequent molecular analyses.

2.2. Chemical and physical soil parameters

Soil pH was analyzed as described in DIN ISO 10390 (International Organization for Standardization, 2005) with the electrode SenTix 61 and pH meter (inoLab pH 720 Level 1, Wissenschaftlich-Technische Werkstätten, Weilheim, DE) in 0.01 M CaCl2 extract of 2 g of fresh soil (Stempfhuber et al., 2015). Nitrate, ammonium, dissolved organic nitrogen (DON) and carbon (DOC), were extracted with 0.01 M CaCl2 solution (in a 1:4 ratio) from 3 g of fresh soil by 45 min overhead shaking at 67 rpm (Reax 2, Heidolph Instruments, Schwabach, Germany), followed by filtration through Whatman™ 595 1/2 filters (Sigma-Aldrich, St. Louis, MO, United States) and determined by a segmented flow analyzer (Skalar SANPlus 5100 with autosampler 1050, Skalar analytic, DE, EU). DOC and DON were analyzed on DIMATOC2000 (DIMATEC Analysentechnik, Essen, DE). To calculate dissolved inorganic carbon (DIC), one drop of 32% HCl was added to the filtrate prior to analysis and the difference to DOC without HCl was used to calculate DIC (Brankatschk et al., 2011). Chlorophyll *a* was used as a proxy for phototrophic biomass and determined according to Ritchie (2008). The procedure was as follows, 0.7 g frozen soil was extracted in 3 mL 96% aqueous ethanol (v/v) and incubated for 30 min at 78°C. Afterwards, the extract was centrifuged at 5°C at 6,000 rpm. The absorption of the supernatant was measured at wavelengths of 632 nm, 649 nm, 665 nm, 696 nm using the spectrophotometer UV-2401PC (Shimadzu, Kyoto, JPN). Extraction was repeated until no chlorophyll could be detected in the supernatant anymore and the content was summed up for all extraction steps.

2.3. DNA extraction

DNA was extracted from 0.3 g of soil using the NucleoSpin® Soil kit (Macherey-Nagel, Düren, DE) according to the manufacturer's manual using lysis buffer SL2 and 150 µL of enhancer. DNA quality was assessed using a UV-VIS spectrophotometer (NanoDrop® ND-1000 spectrophotometer, Thermo Fisher Scientific, Waltham, Massachusetts, United States). DNA concentration was determined using the Quant-IT™ Picogreen® dsDNA Assay Kit (Thermo Fischer Scientific). A negative extraction control sample without soil was processed as negative control to check for possible contaminations of chemicals from the kit used for nucleic acid extraction.

2.4. Quantification of prokaryotic and fungal biomass

Real-time qPCR was used to determine the abundance of Bacteria, Archaea and Fungi, which was used as a proxy for their biomass. For Bacteria (Bach et al., 2002) and Archaea (Bano et al., 2003;

Nicol et al., 2003), kingdom specific primers targeting the V5-V6 (Bacteria) and V2-V5 (Archaea) region of the 16S rRNA gene were used to estimate their absolute abundance separately. For Fungi, primers targeting the ITS1 & 2 region were used (White et al., 1990). SYBR Green® based assays (Applied Biosystems, Foster City, CA, United States) were performed on a 7,300 Real-Time PCR System (Applied Biosystems). Details about forward (F) and reverse (R) primer, reaction conditions, and calibration standards are summarized in Supplementary Table S1. Primers were purchased from Metabion (Planegg, Germany) and bovine serum albumin (BSA) from Sigma-Aldrich (Missouri, United States). To exclude inhibitory effects, dilution tests were performed. Standard series (10^6 to 10^2 gene copies μl^{-1}) and samples diluted to 1/32 were included in each run. To check for possible contamination of chemicals used, a negative control without DNA of samples was included, as well. To evaluate the quality of the qPCR, melting curve analyses were performed and randomly chosen samples were checked by electrophoresis on a 1.5% agarose gel. The amplification efficiency was calculated by $\varepsilon = 10^{(-1/\text{slope})} - 1$ with the slope of the standard series, and was 78–84% for all genes. The r^2 of the standard curve was >0.987.

2.5. Diversity of prokaryotes and Fungi

As no specific primers exist, which separately target Bacteria and Archaea for Illumina MiSeq® sequencing (Illumina, San Diego, CA, United States), we used the universal primers Arch0519 F (Klindworth et al., 2013) and Pro 805 R (Herlemann et al., 2011), which were optimized for the simultaneous sequencing of both prokaryotes, Bacteria and Archaea, and target the V4 region of the 16S rRNA gene. For Fungi, the ITS 3 primer mix and ITS 4 primer mix (Tedersoo et al., 2015) were used. Primer sequences are given in Supplementary Table S2. Each assay was set to 25 µL and consisted of NebNext® High-Fidelity 2X PCR Master Mix (New England Biolabs, Ipswich, MA, United States), forward (F) and reverse (R) primer with Illumina overhang (Metabion), 3% BSA (Sigma-Aldrich), DEPC treated water, and 1 µL of DNA (3 ng μl^{-1}). Primers were purchased from Metabion (Planegg, Germany) and bovine serum albumin (BSA) from Sigma-Aldrich (St. Louis, Missouri, United States). PCR conditions are given in Supplementary Table S2. All samples, PCR negative controls and extraction blanks were amplified in triplicates and checked on 1% agarose gel before pooling. PCR clean-up was carried out with Agencourt AMPure XP magnetic beads (Beckman Coulter Life Sciences, Brea, CA, United States) according to the manufacturer's protocol with a DNA to bead ratio of 0.8. A quality check to assess DNA concentration and fragment size was performed on Fragment Analyzer™ Automated CE System (Agilent Technologies, Santa Clara, CA, United States). For multiplexing, Nextera® XT Index Kit v2 (Illumina) was used. Each indexing PCR reaction of 25 µL consisted of 12.5 µL NebNext® High-Fidelity 2X PCR Master Mix (New England Biolabs), 2.5 µL of indexed forward and reverse primer (10 pmol/µl) and 10 ng of purified amplicon. The following PCR conditions were used: 30 s at 98°C, followed by 8 cycles of 10 s at 98°C, 30 s at 55°C and 30 s at 72°C and 5 min final elongation at 72°C. PCR clean-up was performed with AMPure Beads (Beckman Coulter Life Sciences) as described before. Both DNA concentration and quality were assessed on the Fragment analyzer (Agilent Technologies). Samples were diluted to 4 nM and 5 µL of each library were pooled for sequencing on the MiSeq® instrument with 2 × 300 bp (Illumina) using MiSeq Reagent kit v3 (600 cycles) and spiked with 20% PhiX as a control. Samples with less than 10,000 reads were re-sequenced.

Sequencing adapters were removed using AdapterRemoval (Schubert et al., 2016). DADA2 package Version 1.8.0 (Callahan et al., 2016) in R Version 3.6.1 (R Core Team, 2022) was used for length and quality filtering including PhiX and chimera removal. For prokaryotes, forward and reverse reads were trimmed at 10 and 250 bp and 10 and 200 bp, respectively. For Fungi, the parameters were set to 10 and 275 bp and 10 and 225 bp, respectively. Alignment of mate pairs, inferring into amplicon sequencing variants (ASVs) and merging of sequencing runs was done prior to taxonomy assignment. For prokaryotes, this was done against the Silva database Version 132 (Quast et al., 2013) and for Fungi, against the Unite database Version 7.1 [2016-11-20, Kõljalg et al. (2013)]. After taxonomy assignment, reads present in samples of blank extraction or PCR negative controls were removed (11 of 9,637 ASVs for prokaryotes, 6 of 3,715 ASVs for Fungi) as well as ASVs assigned as Chloroplasts or Mitochondria (on average 11.99%). To differentiate between Chloroplast sequences (representative chloroplast reads from plants, Algae and Bacteria) and cyanobacterial ASVs detected in our study, a phylogenetic tree was calculated and only sequences clearly assigned as cyanobacteria were processed (Supplementary Figure S2). Random subsampling was performed to the lowest number of reads (prokaryotes: 19,722 reads, Fungi: 22,731 reads) using the function rarefy_even_depth() of the R package phyloseq Version 1.30.0 (McMurdie and Holmes, 2013) with the random seed set to 3,006. As they were no longer present after subsampling, 448 ASVs out of 9,626 ASVs for prokaryotes and 283 ASVs out of 3,709 ASVs for Fungi have been removed.

The sequence data was submitted to NCBI via the Sequence Read Archive (SRA) and is available under the accession number PRJNA646655.

2.6. Statistical data analysis

Data analysis was performed with R Version 3.6.1 (R Core Team, 2022). The vegan package Version 2.5-7 was used to calculate rarefaction curves, alpha diversity [Shannon diversity (S) (Shannon, 1948), richness as number of ASVs (R) and Pielou's eveness (P) (Pielou, 1966)] of the subsampled data. To test for the normal distribution of the data residual vs. fitted plots and sample quantile vs. theoretical quantile plots were tested for normal distribution and homogenous variance to verify the models. If needed, data was transformed to meet normal distribution with the 1 + log transformation for abiotic parameters, gene abundances, and alpha diversity; square root transformation for taxa on family level with abundances of more than 2% for at least one replicate of Bacteria and 3% of Fungi. Linear models were applied to detect significant differences according to biocrust presence or treatment for soil parameters, absolute abundances of microbial groups, alpha diversity (S, R, P) and relative abundance of microbial families obtained from sequencing results. To disentangle treatment effects (tillage, mineral fertilization amount, and organic treatment), biocrust and bare soil samples were separated.

Difference in β-diversity of the treatments was calculated by Bray-Curtis-distance, plotted as nonmetric multidimensional scaling (NMDS) plots (Oksanen et al., 2017) and tested for significance by PERMANOVA. The barplots and heatmaps were created using ggplot2 package Version 3.3.5 (Wickham, 2009). CoNet (Faust and Raes, 2016) as an add-on of Cytoscape Version 3.7.2 (Shannon et al., 2003) was used to calculate correlation networks on relative abundance data. For network calculations, ASVs unclassified at the phylum, class and order level were removed. Relative abundance was calculated separately for prokaryotes and Fungi and data sets were merged afterwards. Networks were calculated separately for the two sample (biocrust and bare soil) and management types (tillage: cT and rT, mineral fertilizer: 120 and 240, organic treatment: -org and +org) to detect differences in co-occurrence patterns ($N = 12$). To obtain correlations only valid for all treatments, ASVs present in less than 10 samples were excluded from analysis. Significant correlations (co-occurrences vs. mutual exclusion) were determined based on Pearson and Spearman correlation as well as Bray-Curtis and Kulback-Leibler dissimilarity. At least two of the analyses need to support the link connecting two nodes. 1,000 permutations and bootstrap scores were generated with Brown value of p merging (Brown, 1975). Gephi 0.9.2 (Bastian et al., 2009) was used for visualization of undirected networks with the Fruchterman-Reingold layout (Fruchterman and Reingold, 1991). Correlation partners are visualized with the chordDiagram function of circlize package Version 0.4.13 in R (Gu et al., 2014). Network hubs were defined as highest connected nodes (Tipton et al., 2018), where hubs not specified at the family level and lower were ignored.

3. Results

3.1. Basic properties of biocrusts

The content of phototrophic biomass measured as chlorophyll *a* was 6-times higher in biocrusts compared to bare soil ($F = 183.7$, $p < 0.001$) reaching $17.87 \pm 7.2\,\mu g\,g^{-1}$ soil dry weight (dw) in biocrusts compared to $2.98 \pm 1.2\,\mu g\,g^{-1}$ dw in bare soil (Figure 2; Table 1; Supplementary Table S3). The management had no significant influence on the chlorophyll *a* content. The microbial biomass, determined as the amount of extracted DNA ($F = 18.5$, $p < 0.001$) and the abundance of Bacteria, Archaea and Fungi was significantly higher ($18.7 \geq F \leq 106.8$, $p < 0.001$) in biocrusts compared to bare soils (Supplementary Table S4). Bacterial 16S rRNA genes ($2.3*10^{10} \pm 8.4*10^{9}$ copies g^{-1} dw) were significantly ($F = 277$, $p < 0.001$) more abundant, than Fungal ITS sequences ($2.4*10^{9} \pm 1.3*10^{9}$ copies g^{-1} dw) and archaeal 16S rRNA genes ($1.1*10^{7} \pm 8.9*10^{6}$ copies g^{-1} dw) (Figure 3; Table 1; Supplementary Table S5). In biocrusts, the bacterial and fungal abundance was not influenced by management. Contrastingly in bare soils, tillage, mineral fertilizer amount and organic treatments altered archaeal, bacterial and fungal biomass. For example, in bare soils fungal biomass was significantly higher with organic treatments ($F = 6.7$, $p = 0.02$). Further, an interacting treatment effect for mineral fertilizer and organic treatments were observed and in samples with 120 kg N ha^{-1} $yr.^{-1}$ the fungal abundance was significantly reduced with organic treatment ($F = 6.1$, $p = 0.025$). Although, a similar pattern was observed for bacterial abundance this was not significant

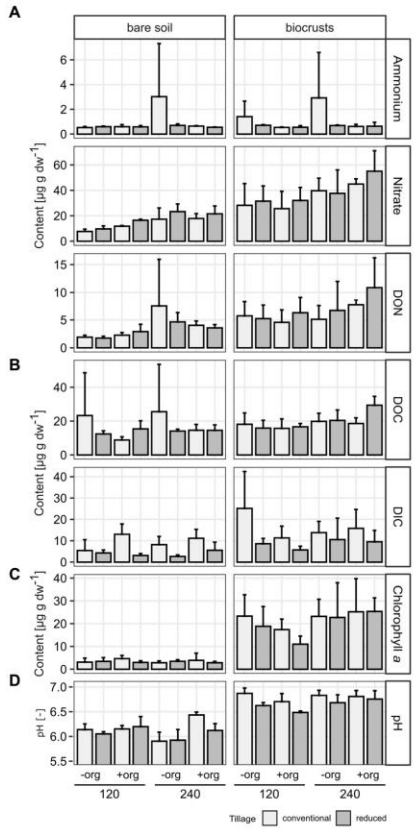


FIGURE 2
Soil properties of **(A)** nitrogen [ammonium, nitrate, and dissolved organic nitrogen (DON)] and **(B)** carbon [dissolved organic (DOC) and inorganic (DIC) carbon] pools, **(C)** chlorophyll a [µg per g soil dry weight (dw)], and **(D)** pH are given as mean values as the average of replicates ($n = 3$) in bar plots with error bars given as standard deviation. Treatments are given for tillage [conventional (cT) vs. reduced tillage (rT)], organic treatment [without (–org) vs. with crop residues (+org)], and mineral fertilization amount (120 vs. 240 kg N/ha·a).

($F = 3.6$, $p = 0.059$). Archaeal biomass was reduced in biocrusts and bare soils in the treatment with mineral fertilizer of 240 kg N ha^{-1} $yr.^{-1}$ ($F > 10.8$, $p \leq 0.05$).

The development of biocrusts came along with changes in abiotic soil properties. In general, water content, ($F = 7.78$, $p < 0.001$; biocrusts $13.7\% \pm 2.8$, bare soils $11.6\% \pm 2.1$), pH (6.71 ± 0.2), nitrate ($36.79 \pm 12.7\,\mu g\,g^{-1}$ dw), DON ($6.52 \pm 3.0\,\mu g\,g^{-1}$ dw), DOC ($19.3 \pm 4.8\,\mu g\,g^{-1}$ dw) and DIC ($12.53 \pm 7.1\,\mu g\,g^{-1}$ dw) were up to 2.4 times higher ($236.9 > F > 5.5$, $p < 0.03$) in biocrusts compared to bare soils (Figure 2; Table 1; Supplementary Table S3). Only ammonium values were similar ($p = 0.475$) in biocrusts ($1.004 \pm 0.7\,\mu g\,g^{-1}$ dw) and bare soils ($0.898 \pm 0.6\,\mu g\,g^{-1}$ dw) but revealed very high standard deviations. In biocrusts, cT further increased pH (6.80) and DIC

TABLE 1 *F* and *p* values of linear models for the effect of biocrust presence, as well as separated for both compartments (bare soil, biocrust) the effect of tillage (cT vs. rT), mineral fertilizer amount (120 vs. 240) and organic treatment (−org vs. +org) as well as their interacting effects on 1+log transformed values of soil properties [ammonium, nitrate and dissolved organic nitrogen (DON), dissolved organic (DOC) and inorganic (DIC) carbon], chlorophyll *a*, pH and abundances of Archaea, Bacteria, and Fungi determined by qPCR.

Variable	Biocrust effect		Compartment	Tillage		Mineral fertilizer amount		Organic treatment		Tillage * organic treatment		Tillage * mineral fertilizer amount		Mineral fertilizer amount * organic treatment		Tillage * mineral fertilizer amount * organic treatment	
	F value	*p* value		*F* value	*p* value	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value	*F* value	*p* value
Ammonium	0.5	0.475	Bare soil	0.54	0.472	1.48	0.241	0.88	0.363	0.14	0.714	1.07	0.317	1.27	0.276	0.45	0.514
			Biocrust	1.98	0.178	0.39	0.541	5.03	**0.039**	1.74	0.205	0.23	0.637	0.04	0.845	0.18	0.679
Nitrate	57.5	**<0.001**	Bare soil	6.25	**0.024**	27.18	**0.000**	5.44	**0.033**	0.02	0.884	0.01	0.931	5.11	**0.038**	0.39	0.541
			Biocrust	0.60	0.450	5.74	**0.029**	0.52	0.483	0.26	0.620	0.43	0.520	0.67	0.425	0.08	0.780
DON	17.2	**<0.001**	Bare soil	0.03	0.874	12.98	**0.002**	0.05	0.832	0.11	0.749	0.17	0.688	1.85	0.192	0.13	0.723
			Biocrust	0.28	0.602	1.08	0.313	1.42	0.250	0.50	0.490	0.00	0.993	1.75	0.205	0.03	0.869
DOC	5.5	**0.025**	Bare soil	0.02	0.904	0.54	0.472	0.37	0.550	1.12	0.305	0.28	0.606	0.07	0.801	0.41	0.529
			Biocrust	1.05	0.320	6.32	**0.023**	0.33	0.574	2.20	0.157	1.25	0.281	0.81	0.382	0.27	0.608
DIC	10.1	**0.003**	Bare soil	12.99	**0.002**	0.35	0.565	3.98	0.063	1.82	0.196	0.32	0.579	0.01	0.914	3.79	0.069
			Biocrust	5.50	**0.032**	0.02	0.879	0.19	0.667	0.35	0.562	0.01	0.912	1.88	0.190	0.01	0.938
Chlorophyll *a*	183.7	**<0.001**	Bare soil	0.11	0.746	0.19	0.665	0.22	0.645	1.55	0.230	0.39	0.540	0.48	0.498	0.08	0.778
			Biocrust	0.90	0.357	2.55	0.130	0.42	0.528	0.00	0.965	0.81	0.380	1.59	0.225	0.39	0.539
pH	236.9	**<0.001**	Bare soil	1.89	0.188	0.50	0.488	13.80	**0.002**	0.67	0.425	1.05	0.322	5.71	**0.029**	3.66	0.074
			Biocrust	11.25	**0.004**	3.85	0.067	1.67	0.214	0.35	0.562	1.75	0.205	3.21	0.092	0.14	0.716
Archaea	18.7	**<0.001**	Bare soil	0.54	0.472	10.83	**0.005**	3.67	0.073	6.40	**0.022**	1.97	0.180	0.46	0.506	4.52	**0.049**
			Biocrust	0.33	0.571	11.71	**0.003**	0.06	0.817	0.00	0.990	0.94	0.347	0.00	0.976	0.00	0.998
Bacteria	99.1	**<0.001**	Bare soil	4.95	**0.041**	6.25	**0.024**	3.55	0.078	4.15	0.059	0.62	0.444	0.00	0.983	8.89	**0.009**
			Biocrust	0.91	0.356	1.18	0.293	2.92	0.107	0.92	0.351	0.20	0.658	1.23	0.283	0.20	0.662
Fungi	106.8	**<0.001**	Bare soil	1.01	0.329	0.26	0.616	6.65	**0.020**	3.35	0.086	6.66	**0.020**	6.08	**0.025**	6.48	**0.022**
			Biocrust	0.00	0.983	1.23	0.284	3.06	0.099	1.19	0.292	0.28	0.604	0.11	0.748	0.17	0.685

Significant values ($p < 0.05$) are marked in bold.

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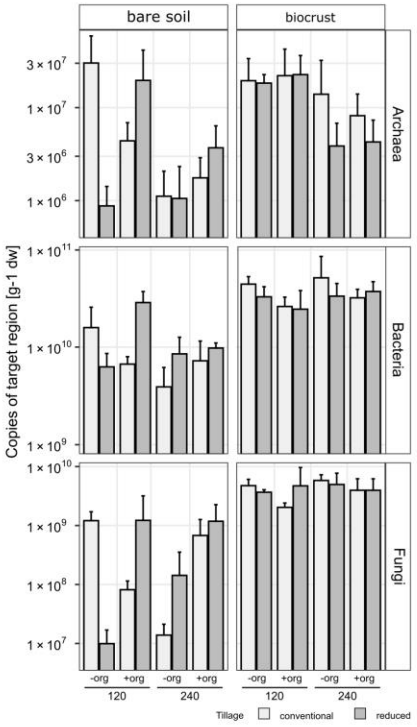

FIGURE 3
Copies of target region [per gram soil dry weight (dw)] 16S rRNA gene for Archaea and Bacteria and ITS region for Fungi in bar plots as the average of replicates (n=3) and error bars displaying the standard deviation. Treatments are given for tillage [conventional (cT) vs. reduced tillage (rT)], organic treatment [without (–org) vs. with crop residues (+org)] and mineral fertilization amount (120 vs. 240 kg N/ha·a).

($16.51\,\mu g\,g^{-1}$ dw) compared to rT (6.34 and $8.56\,\mu g\,g^{-1}$ dw), while higher mineral N fertilization increased nitrate (29.28–$44.30\,\mu g\,g^{-1}$ dw) and DOC (16.57–$22.03\,\mu g\,g^{-1}$ dw) concentrations significantly ($F>5.5$, $p<0.003$). In bare soils, pH increased due to organic treatments (6.00–6.23), while nitrate (11.33–$20.24\,\mu g\,g^{-1}$ dw) and DON (2.20–$6.08\,\mu g\,g^{-1}$ dw) increased significantly ($F>13$, $p<0.002$) due to higher mineral fertilization. Only in bare soils, the interaction of mineral fertilization amount and organic treatments had significant effects ($F>5.1$, $p<0.04$) on nitrate and pH (Figure 2; Table 1).

3.2. Microbial diversity in biocrusts

In total, 3,601,616 reads were obtained from 16S rRNA gene amplicon sequencing and 4,995,629 reads from ITS amplicon sequencing. The average loss of reads because of the different filter steps during data processing was 18.9% for prokaryotes and 29.4% for Fungi (Supplementary Tables S6, S7). However, after subsampling, all rarefaction curves still reached a plateau indicating a sufficient sampling depth for further analysis (Supplementary Figure S3). Details of all sequencing runs including number of demultiplexed, filtered and merged reads are summarized in the Supplementary Tables S6, S7.

For α-diversity indices of prokaryotes, no difference between bare soils and biocrusts was detected ($F<0.6$, $p>0.4$). In contrast, fungal diversity and richness were significantly ($F>8.8$, $p<0.005$) lower in biocrusts ($S=3.97\pm0.35$, $R=274\pm88$) compared to bare soils ($S=4.35\pm0.43$, $R=390\pm110$), while evenness was also not affected. Regarding the management, the evenness of prokaryotes was reduced in cT compared to rT in both compartments ($F>6$, $p<0.03$) (Supplementary Tables S8, S9), while tillage had contrasting effects on fungal α-diversity in bare soils and biocrusts. For example, rT compared to cT caused a decrease in fungal richness in bare soils ($F=11.9$, $p=0.003$) and an increase in biocrusts ($F>6.6$, $p<0.02$). Interacting treatment effects ($p<0.05$) were detected in biocrusts for archaeal/bacterial richness (Tillage * Mineral Fertililzer Amount * Organic Treatment) and fungal diversity and richness (Tillage * Organic Treatment) (Supplementary Tables S8, S9).

Beta-diversity analysis revealed significant differences between biocrust and bare soil microbial communities ($p<0.02$) as well as between the different treatments ($p<0.05$) (Table 2; Supplementary Figures S4, S5). Regarding the prokaryotic community, the presence of biocrusts reduced treatment effects on the community composition. The fungal community composition was affected by all treatments no matter if biocrusts or bare soils were considered ($p<0.05$).

3.3. Microbial community composition in biocrusts

In total, 2,860 different bacterial and 48 archaeal families were detected, of which 39 bacterial families were highly abundant (at least 2% abundance in one of the samples) (Figure 4B). Predominant families were *Sphingomonadaceae* (bare soils 10.1 ± 1.9%, biocrusts 8.6 ± 1.2%), *Burkholderiaceae* (bare soils 7.0 ± 1.4%, biocrusts 7.2 ± 1.5%), "Unknown Family" of Oxyphotobacteria (bare soils 6.8 ± 4.3%, biocrusts 7.1 ± 3.1%), *Chitinophagaceae* (bare soils 3.6 ± 1.6%, biocrusts 7.1 ± 3.8%), and *Flavobacteriaceae* (bare soils 2.1 ± 1.0%, biocrusts 3.6 ± 1.8%). The most abundant archaeal family was *Nitrososphaeraceae* with 0.34 ± 0.30% of abundance in biocrusts and 0.48 ± 0.3% in bare soils (Figure 4A).

Overall, 19 bacterial families were significantly ($p<0.03$) different between biocrusts and bare soils (Figure 4B). Eleven of those were higher in relative abundance in biocrust samples, including many reads which were assigned as α-and γ-Proteobacteria, while reads corresponding to Acidobacteria and Actinobacteria were lower abundant in biocrusts compared to bare soils. Interestingly, the relative abundance of ASVs linked to cyanobacterial families was similar in biocrusts (16.8 ± 9.0%) and bare soils (16.0 ± 13.3%).

The amount of mineral fertilizer addition significantly ($4.6<F<23.0$, $p<0.05$) changed the relative abundance of 54% of the highly abundant bacterial families in biocrusts and bare soils. The identity of the affected families was mostly the same in biocrusts and bare soils and included the highly abundant *Burkholderiaceae* and *Chitinophagaceae*. Regarding Cyanobacteria, the relative abundance of *Coleofasciculaceae* and *Nostocaceae* was seven times higher in

TABLE 2 Anova Results on Bray-Curtis-Distance-Matrix of Community data on ASV Level.

Kingdom	Compart-ment	Management effect		
			r^2	p
Prokaryotes	Biocrust vs. Bare soil		0.060	0.001
	Biocrust	Tillage	0.123	**0.001**
		Mineral Fertilizer Amounts	0.084	**0.005**
		Organic Treatment	0.043	0.236
		Tillage * Mineral Fertilizer Amounts	0.047	0.138
		Tillage * Organic Treatment	0.047	0.143
		Mineral Fertilizer Amounts * Organic Treatment	0.033	0.542
		Tillage * Mineral Fertilizer Amounts * Organic Treatment	0.046	0.149
	Bare soil	Tillage	0.092	**0.001**
		Mineral Fertilizer Amounts	0.082	**0.007**
		Organic Treatment	0.087	**0.002**
		Tillage * Mineral Fertilizer Amounts	0.032	0.591
		Tillage * Organic Treatment	0.054	0.077
		Mineral Fertilizer Amounts * Organic Treatment	0.030	0.710
		Tillage * Mineral Fertilizer Amounts * Organic Treatment	0.031	0.701
Fungi	Biocrust vs. Bare soil		0.040	0.013
	Biocrust	Tillage	0.155	**0.001**
		Mineral Fertilizer Amounts	0.091	**0.006**
		Organic Treatment	0.097	**0.002**
		Tillage * Mineral Fertilizer Amounts	0.061	**0.049**
		Tillage * Organic Treatment	0.053	0.087
		Mineral Fertilizer Amounts * Organic Treatment	0.025	0.616
		Tillage * Mineral Fertilizer Amounts * Organic Treatment	0.016	0.921
	Bare soil	Tillage	0.100	**0.004**
		Mineral Fertilizer Amounts	0.082	**0.007**
		Organic Treatment	0.101	**0.002**
		Tillage * Mineral Fertilizer Amounts	0.015	0.989
		Tillage * Organic Treatment	0.064	**0.033**
		Mineral Fertilizer Amounts * Organic Treatment	0.033	0.532
		Tillage * Mineral Fertilizer Amounts * Organic Treatment	0.025	0.803

Significant values ($p < 0.05$) are marked in bold.

treatments with 120 kg N ha^{-1} y^{-1} compared to 240 kg N ha^{-1} y^{-1} in both biocrusts and bare soils ($F > 5.8$, $p < 0.02$). In contrast, the effect of crop residues was less pronounced, especially in biocrusts only 10% (compared to 26% in bare soils) of the dominant bacterial families were positively affected and had higher abundances due to the organic treatment. Of those, *Pseudonocardiaceae*, *Xanthobacteraceae*, *Chthoniobacteraceae*, and *Rubritaleaceae* were affected by mineral fertilizer and organic treatmentor the interaction of both (*Pseudonocardiaceae*, *Chthoniobacteraceae*) (Figure 4B).

Tillage significantly ($15.1 < F < 65.3$, $p < 0.04$) changed the relative abundance of 15 bacterial families in biocrusts, but only seven in bare soils. For example, in biocrusts *Chitinophagaceae* were increased by cT (4.7 ± 1.4% to 9.4 ± 4.3%) ($F \geq 23.0$, $p < 0.001$) while the "Unknown Family" of Oxyphotobacteria (including also species of *Leptolyngbya* in Silva database v132) decreased under rT (8.8 ± 3.3% to 5.3 ± 3.7%) ($F \geq 8.0$, $p \leq 0.01$). Interacting treatment effects were mainly observed, where one of the factors alone significantly influenced the abundance of bacterial families (Supplementary Table S10). Only the family *env. OPS_17* (Bacteroida of Bacteroidetes) in biocrusts was significantly ($F = 6.4$, $p = 0.027$) affected by all treatments in combination but not by one single one. *Longimicrobiaceae* in biocrusts were significantly ($F = 6.0$, $p = 0.027$) affected by tillage and in combination with mineral fertilization amount without these two affecting the family individually. In general, more interacting effects were observed in biocrusts ($n = 11$) than in bare soils ($n = 5$).

Two hundred and twenty different fungal families were detected. In our analyses we focused on the 29 most abundant ones (at least 2% abundance in one of the samples) (Figure 4C). Predominant families were *Nectriaceae* (bare soils 22.6 ± 6.9%, biocrusts 23.1 ± 7.2%), *Pleosporaceae* (bare soils 14.4 ± 5.6%, biocrusts 19.4 ± 7.1%),

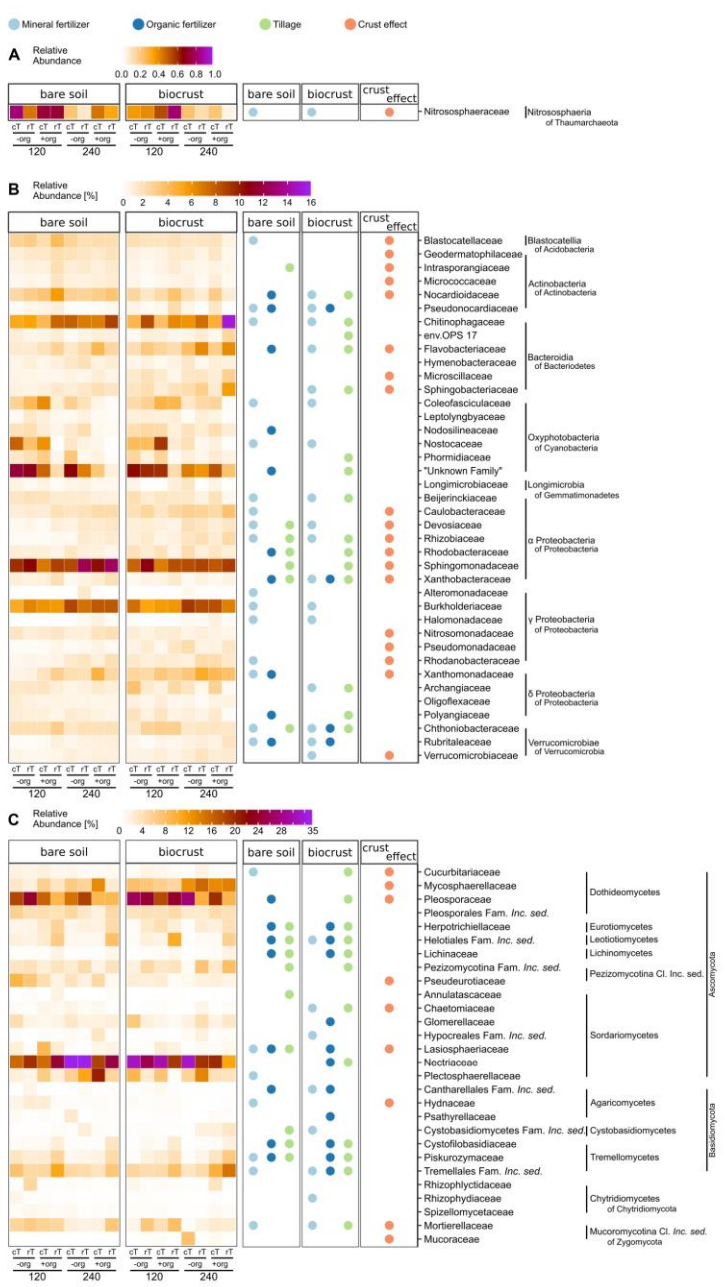

FIGURE 4
Heat maps of **(A)** archaeal, **(B)** bacterial, and **(C)** fungal community composition in bare soils and biocrusts at the family level are displayed in the left two columns (for Bacteria **(B)** and Fungi **(C)** with an abundance above 2% in at least one replicate). The average of field replicates (n = 3) is presented. Values range from white (0%), orange, and dark red to the highest abundance in purple **(A)** 1%, **(B)** 16%, **(C)** 35%). Significant variations ($p < 0.05$) are shown in the right columns for the biocrust effect (bare soil vs. biocrust) (orange), mineral fertilizer amount (light blue), organic treatment (dark blue), and tillage (green) separately for bare soils and biocrusts. The families additionally influenced by interacting treatment effects are supplied in Supplementary Tables S10, S11.

Mycosphaerellaceae (bare soils 4.9 ± 3.6%, biocrusts 9.8 ± 4.1%), Tremellales Fam. *Incerta sedis* (*Inc. sed.*) (bare soils 5.6 ± 2.4%, biocrusts 6.4 ± 3.9%), and *Plectosphaerellaceae* (bare soils 6.5 ± 6.4%, biocrusts 5.3 ± 4.1%). One third of the fungal families significantly ($4.31 < F < 12.1$, $p < 0.05$) differed in relative abundance between biocrusts and bare soils, of which many families were 1.3–4.5 times more abundant in bare soils (Figure 4C). The fungal community in biocrusts was more sensitive toward all management practices than bare soil communities. For example, 44% of the dominant families in biocrusts were significantly influenced by cT, of which the very high abundant families *Nectriaceae* (cT: 27.7%, rT: 18.5%) ($F = 9.1$, $p = 0.008$) and *Pleosporaceae* (cT: 22.4%, rT: 16.3%) ($F = 5.1$, $p = 0.04$) were significantly increased while the others were lower compared to rT. Additionally, +org influenced more fungal families in the biocrusts (39%) compared to bare soils (29%). However, *Herpotrichiellaceae*, Helotiales Family *Inc. sed.*, *Lasiosphaeriaceae*, *Lichinaceae*, Cantharellales Family *Inc. sed.*, *Cystofilobasidiaceae* (in bare soil not significantly), and *Piskurozymaceae* were increased up to six times in both, bare soils ($F > 5.5$, $p < 0.02$) and biocrusts ($F > 4.6$, $p < 0.05$) under +org. Mineral fertilizer alone changed the abundance of the same number of families in biocrusts and bare soils, but their identity mostly differed. Interacting treatment effects were equally distributed in bare soils and biocrusts ($n = 9$) (Supplementary Table S11). In bare soils, Pleosporales Fam. *Inc. sed.*, Tremellales Fam. *Inc. sed.* and *Mucoraceae* were affected by tillage combined with organic treatment, and for Cystobasidiomycetes Fam. *Inc. sed.* of mineral fertilizer and organic treatment combined was observed, without the single effects influencing those fungal families (Supplementary Table S11).

3.4. Cross-kingdom correlation networks in biocrusts

Network analysis represents co-occurrence and mutual exclusion patterns of the microbial community based on significant correlations (edges) between taxa (nodes) detected in >80% of the samples. Networks were calculated separately for both compartments (biocrusts and bare soils) and management effects (cT vs. rT, 120 vs. 240 N kg ha^{-1} yr.$^{-1}$, −org vs. +org), resulting in 12 correlation networks based each on 12 samples given in co-occurrence and mutual exclusion (Figures 5, 6). The total number of nodes (in average for bare soils: 209, biocrusts: 152) and edges (bare soils: 367, biocrusts: 238) was higher in bare soils for all treatments (Table 3). Especially rT resulted in the highest numbers of edges and nodes in bare soils reaching 645 and 255, respectively. The same positive effect of rT on the number of edges and nodes was observed for biocrusts (nodes: 223, edges: 417). In biocrusts, the detrimental effect of cT were most pronounced as the number of edges and nodes dropped 1.5 times more compared to bare soils. Regarding the nature of the correlations (co-occurrence vs. mutual exclusion) an interesting pattern emerged based on the comparison of fertilization treatments and tillage: biocrusts had a higher proportion of co-occurrences compared to bare soils in all treatments related to mineral fertilization or organic treatment peaking in the treatments with highest application rates like 240 (77.5%) and +org (76.8%). In contrast, rT and cT networks revealed a higher proportion of co-occurrences in bare soils with the highest value found under rT (77.3%).

Bacteria were the dominating kingdom in our network analysis forming up to 82% of nodes. In 11 of 12 networks, the ratio of nodes assigned to Bacteria and Fungi ranged between 2 and 3.6, with cT being the only exception. Here, the Bacteria:Fungi ratio dropped to 1.4 in bare soils, while it strongly increased in biocrusts to a ratio of 5. This could be assigned to a reduction of Tremellomycetes and Dothideomycetes ASVs in the network. With the increasing share of Fungi in the bare soil network, bacterial-fungal correlations increased under cT, mostly between different Basidiomycota and Actinobacteria, Oxyphotobacteria and Phycisphaerae.

In total, 31 different families of Archaea, Bacteria and Fungi in biocrusts and 25 in bare soils were identified as network hubs (highest degree), where 12 families appeared in both compartments as hubs (Figures 5, 6, highlighted in bold in Supplementary Table S12). Out of the 31 hub families, 19 and 13 were specific in biocrusts and bare soils, respectively. Most of these unique hubs only appeared in one or two networks. The only exceptions of the specific families were *Blastocatellaceae* in bare soils, which were identified as hubs in 240, −org, cT, and rT networks and *Rhodobacteraceae* in biocrusts, which formed hubs in networks 120, −org, +org, and cT.

4. Discussion

4.1. Biocrusts are richer in nutrients and microbial abundance than bare agricultural soils

Biocrusts have previously been described in arid or oligotrophic environments where the accumulation of nutrients is one of their characteristic properties (Brankatschk et al., 2013; Schulz et al., 2013; Maier et al., 2018). To investigate whether this also holds true in fertilized, mesic agricultural ecosystems was one major aim of this study. We observed that under sugar beet cultivation in mesic regions, biocrusts could form large patches (Figure 1; Supplementary Figure S1) with high contents of phototrophic biomass, which was comparable to those of arid biocrusts (Román et al., 2019). Biocrust formation was accompanied by increased DOC, nitrate and DON concentrations as well as higher microbial biomass compared to bare soil implying that these typical biocrust properties are also present in these mesic biocrusts. However, we cannot exclude that this effect changes over the course of a season or at different soil types, which retain nutrients better than these poor soils with high amounts of sand (Kurth et al., 2021). The increase in nutrient concentrations might be attributed to two different mechanisms. First, due to the activity of the phototrophic biomass, CO_2 is fixed and increased DOC concentrations. This is supported by the positive correlation of DOC (Supplementary Figure S6) and chlorophyll *a* concentration in the biocrusts. Additionally, the significantly higher relative abundance of potential N_2 fixing bacteria like *Rhizobiaceae* might further facilitate nitrogen input as shown for other biocrusts previously (Lan et al., 2011; Maier et al., 2018; Román et al., 2019). Second, the polymeric matrix of biocrusts traps or retains nutrients more efficiently (Costa et al., 2018; Rossi et al., 2018; Cania et al., 2019a). This is corroborated by the higher relative abundance of Bacteria potentially involved in the production of extracellular polysaccharides in the biocrusts like *Burkholderiaceae*, *Flavobacteriaceae*, *Chitinophagaceae*, *Leptolyngbyaceae*, and *Rhizobiaceae* (Cania et al., 2019a; Vuko et al., 2020).

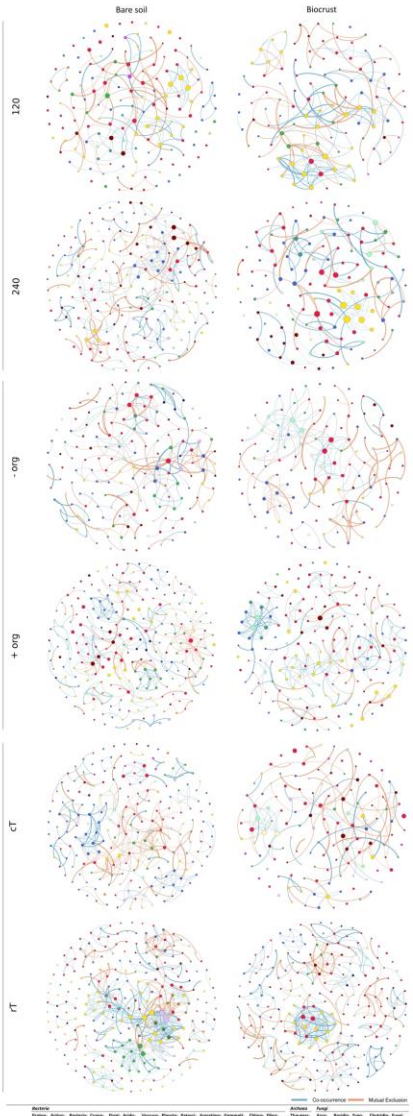

FIGURE 5
Networks in Fruchterman-Reingold layout based on significant ASV correlation analysis for bare soil and biocrust for each management variation (n=12). Nodes are colored by phylum and the color code is given below the network figures, where also the abundance of phyla within the networks is given. Red connections display negative and green connections display positive correlations. The number of correlations defines the size of one node. The lower the p value of the correlation, the thicker the line between two nodes.

4.2. Biocrusts have less diverse microbial communities but promote co-occurrences across kingdoms

Although microbial biomass and nutrient concentrations were generally higher in biocrusts, alpha-diversity and network density were lower (Figures 3, 5, 6; Supplementary Tables S6, S7). These findings contradict previous biocrust studies, where microbial diversity increased with ongoing biocrust development (Chilton et al., 2018; Maier et al., 2018). However, these studies were conducted in arid or nutrient scarce environments, where biocrusts served as habitable micro-ecosystems and paved the way for the attraction of further microbes. In line with our finding, Glaser et al. (2022b) also found reduced diversity in nutrient-poor, mesic dunes. This data, as well as ours from agricultural biocrusts of mesic regions, support findings from other nutrient-rich hotspots in managed ecosystems like the rhizosphere, drilosphere (Uksa et al., 2015) and biocrusts from mesic forests (Glaser et al., 2022a), where only a subset of the diverse bulk soil community was selected by the specific hotspot. Comparable to those hotspots, correlation networks were dominated by *Sphingomonadaceae* (α-Proteobacteria), *Burkholderiaceae* (γ-Proteobacteria), *Chitinophagaceae* (Bacteroidia), *Nocardioidaceae* (Actinobacteria), and *Pleosporaceae* (Dothideomycetes) (Supplementary Table S12). These families were described as key microbiota during organic matter decomposition (Banerjee et al., 2016), particularly the degradation of chitin and cellulose (Coenye, 2014; Glaeser and Kämpfer, 2014; Maier et al., 2018; Wieczorek et al., 2019). Thus, they may be advantageous for organic C turnover in biocrusts, which were introduced by organic treatment or activities of phototrophic microbes. Furthermore, *Sphingomonadaceae*, *Burkholderiaceae* and *Chitinophagaceae* or *Pleosporaceae* and *Nectriaceae* were further detected as dominating families in exo-and lipopolysaccharides production, especially in developing biocrusts, during soil formation and under conventional tillage (Osińska-Jaroszuk et al., 2015; Xiao and Zheng, 2016; Cania et al., 2019a,b; Vuko et al., 2020). Actinobacteria were important nodes in biocrust networks. However, they were not among the high abundant taxa, which highlights that rarely abundant taxa may contribute essential functions to microbial communities (Shi et al., 2016; Benjamino et al., 2018). Indeed, they were correlated to other proteo-, cyanobacterial or fungal classes but most remarkably was the high proportion of their self-co-occurrences. Though, they share the same niches and promote the growth of other groups of their own phylum (Berry and Widder, 2014; de Vries and Wallenstein, 2017). The actinobacterial network participation was much less under conventional tillage compared to reduced tillage. We observed a drop of 30.7% in bare soils and of 11.6% in biocrusts (see Figures 5, 6). Disturbances were shown to change network composition (Berga et al., 2012) and tillage was shown to affect Actinobacteria due to their hyphal growth form (Cania et al., 2020). Very interesting is that this drop is much lower in biocrusts, which is why we conclude that Actinobacteria are somehow protected against tillage in biocrusts. Important functions covered by Actinobacteria might be their ability to fight fungal infections in crops (Barka et al., 2016) or the degradation of pesticides (Mawang et al., 2021). Further functional analysis would be necessary to answer questions about their beneficial potential in agriculture. Nevertheless, this finding opens the debate for further research about the potential and risks of biocrusts in agricultural ecosystems.

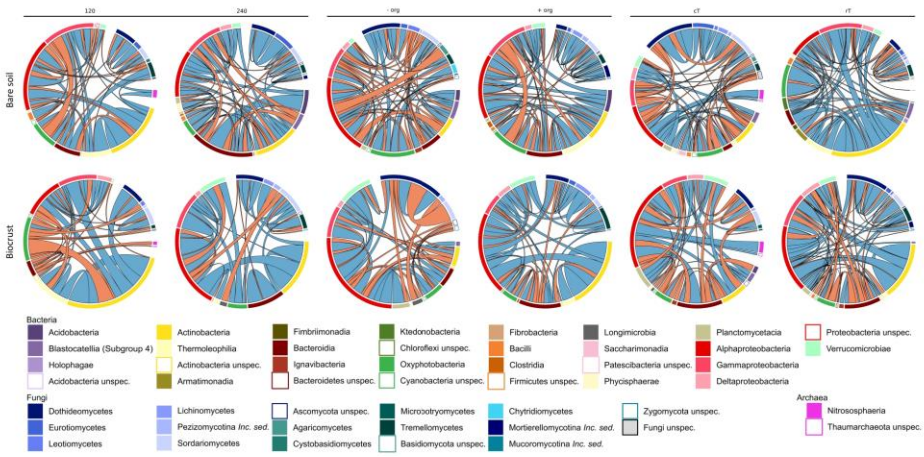

FIGURE 6
Correlation partners on class level based on the network analysis (n=12) are shown as shares on total edges (in %) for bare soils and biocrusts for each management variation [mineral fertilization amount (120 vs. 240 kg N/ha·a), organic treatment (−org vs. +org), and tillage (conventional (cT) vs. reduced (rT) tillage)]. Red connections display negative and green connections display positive correlation.

Despite the lower complexity of biocrust correlation networks, the share of co-occurrences was higher in biocrusts compared to bare soil. The polymeric matrix of biocrusts facilitates not only nutrient trapping but also the exchange among microbes by, for example, facilitating movement and horizontal gene transfer interaction among microbes (Costa et al., 2018; Rossi et al., 2018; Cania et al., 2019a). Moreover, the complexity of extracellular polysaccharides and proteins promotes the association of microbes, which can decompose polymers (Rossi et al., 2018). This is further underlined in our study by the finding that the highest frequency of co-occurrences is in the +org treatment, which adds further complex and recalcitrant carbon compounds to the biocrusts (Wang et al., 2017). Indeed, it could be demonstrated earlier that addition of organic treatment increases network complexity in soil (Schmid et al., 2018). The organic amendment specifically increased co-occurrences of bacterial-fungal partners (Figures 5, 6) like Alphaproteobacteria, Dothideomycetes, and Pezizomycotina, which had been identified as keystone taxa in organic matter decomposition previously (Hartmann et al., 2015).

4.3. Differential response of Cyanobacteria in bare soil and biocrusts

Cyanobacteria belonged to the dominating Bacteria (Figures 4, 5)—surprisingly, not only in the biocrusts but also in bare soils. This contradicts other agricultural studies where they have not been detected or only displayed a minor proportion of the bulk soil community (Uksa et al., 2015; Cano-Díaz et al., 2019). In particular, *Microcoleus* was one of the abundant observed genera in our study. *Microcoleus* is known as a key taxon for biocrust formation in drylands due to bundle sheath and the production of sticky extracellular polymeric substances (Garcia-Pichel and Wojciechowski, 2009; Pereira et al., 2013) and was shown to attract copiotrophic microbes by releasing photosynthesized carbon into the cyanosphere (Couradeau et al., 2019). This was further accompanied by chlorophyll *a* concentration in the bare soils in the same range as detected for Cyanobacteria-dominated biocrusts in arid areas (Román et al., 2019). Also, we identified Cyanobacteria as network hubs with a proportion of up to 12.5% of all network edges in bare soil networks (Supplementary Table S12), which suggests an early crustal stage, as observed in other early-stage biocrusts (Chilton et al., 2018; Maier et al., 2018). These connections might be beneficial for copiotrophic Bacteria (*Burkholderiaceae*, *Rhodobacteraceae*) and Fungi (*Cucurbitariaceae*), when no additional organic carbon was provided by crop residues (Couradeau et al., 2019; Pombubpa et al., 2020). In contrast, bare soil treatments with organic fertilization promoted negative correlations between Cyanobacteria and Gammaproteobacteria, Dothideo-and Sordariomycetes (Mueller et al., 2015; Wen et al., 2020), which might be attributed to the growth of heterotrophic Bacteria responding to the carbon input (Fierer et al., 2007).

Interestingly, the biocrusts of the cT treatment and the bare soils show similar network pattern with highest cyanobacterial relative abundance and connectivity (Figures 4–6; Supplementary Table S10). As revealed by different studies, soil surface disturbance, like tillage, is a critical factor for biocrust development and sets biocrusts back to an initial development stage, where Cyanobacteria play a crucial role (Lange et al., 1997; Kuske et al., 2012; Bu et al., 2013; Ferrenberg et al., 2015; Steven et al., 2015; Belnap et al., 2016; Maier et al., 2018). Biocrusts that were destroyed by soil turning due to tillage before seeding, quickly re-establish with a fast succession. This regeneration of biocrusts after soil surface disturbance observed in this study

TABLE 3 Summary of network parameters including number of nodes and edges per kingdom or between kingdoms and the share of co-occurrences and mutual exclusions.

		Bare soil						Biocrust					
		120	240	−org	+org	cT	rT	120	240	−org	+org	cT	rT
Total number of ASVs (network input)	Total	4,909	5,438	5,203	5,126	5,189	4,881	4,657	4,395	4,248	4,912	4,172	4,725
	Bacteria	3,941	4,659	4,307	4,320	4,192	4,228	4,122	3,858	3,753	4,357	3,757	4,115
	Archaea	18	11	13	17	15	10	18	13	11	19	14	15
	Fungi	950	768	883	789	982	643	517	524	484	536	401	595
Number of nodes	Total	155	214	163	248	219	255	111	130	128	181	139	223
Shares [%]	Bacteria	77.42	77.57	66.87	75	56.62	81.57	75.68	73.08	74.22	76.24	79.86	71.3
	Archaea	1.29	0	0	0	2.74	0.39	3.6	0	0	0	4.32	0
	Fungi	21.29	22.43	33.13	25	40.64	18.04	20.72	26.92	25.78	23.76	15.83	28.7
Number of edges	Total	193	348	233	448	326	645	173	183	172	320	160	417
Shares [%]	Bacteria - Bacteria	74.6	76.7	48.5	79.9	41.7	88.2	77.5	68.3	63.4	67.2	74.4	69.8
	Archaea - Archaea	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Fungi - Fungi	11.4	17.5	10.3	17.2	25.8	9.6	7.5	16.9	17.4	11.9	6.3	15.6
	Bacteria - Archaea	4.2	0.0	0.0	0.0	6.1	0.2	2.3	0.0	0.0	0.0	7.5	0.0
	Fungi - Archaea	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Bacteria - Fungi	9.8	5.8	41.2	2.9	26.4	2.0	12.7	14.8	19.2	20.9	11.9	14,6
	Co-occurrence	58.2	63.5	51.2	60.4	50.7	77.3	62.9	77.5	50.0	76.8	44.0	65.8
	Mutual exclusion	41.8	36.5	48.8	39.6	49.3	22.7	37.1	22.5	50.0	23.2	56.0	34.2

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happened much faster than observed in (semi-) arid areas (Weber et al., 2016), where recovery rarely takes less than 5 years, and can need several decades depending on the type of disturbance. Nevertheless, to disentangle specific patterns of biocrust establishment on mesic, managed ecosystems, future studies need to analyze disturbance effects in more detail, in more systems and throughout the year.

4.4. Conclusion

Our study demonstrates that despite, and sometimes because of, intensive management like tillage and fertilization, biocrusts develop on agricultural fields and build up substantial phototrophic biomass over one growing season. Increased retention of nutrients and water-mediated by biocrusts might improve water and nutrient availability at the beginning of the crop-growing season, possibly with positive feedback on crop growth. This was associated by a reduction of microbial diversity and the promotion of cross-kingdom co-occurrences, indicating that biocrusts become an additional hotspot for activity in soils comparable to rhizosphere or drilosphere. Surprisingly, Cyanobacteria played a negligible role in biocrust networks in autumn before harvest but dominated networks in bare soil communities. Thus, we conclude that they may serve as a seed for biocrust formation, at least in agricultural soils with low quality in terms of organic matter content or waterholding capacity. However, the effect of tillage on the biocrust formation pattern and biocrust properties requires further investigation. Future studies should be accompanied by measurements recording seasonal changes or resilience measurements toward short-term effects like summer drought or heavy rain events.

Data availability statement

The sequencing datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Material and Methods. Raw data for soil properties generated and analyzed for this study are available from the corresponding author upon request.

Author contributions

SS, MS, and UK contributed to the conceptualization and design of this study. MAr manages the fields of LUFA Speyer and assisted in sampling preparation and performance. Sampling was performed by SS. Material preparation, data collection, and analysis were performed by JK. Sequencing was performed by SK. The pipeline for quality filtering of sequencing and taxonomic assignment was provided by CS. The pipeline for network analysis was provided by GV. The first version of the manuscript was written by JK and all authors commented on previous versions of the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmicb.2023.1169958/full#supplementary-material

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Supplementary Material

1. Tables

Table S1 Primer, reaction mixture, conditions and calibration standards for real-time qPCR.

Target Region	Reaction conditions thermal profile	No. of Cycles	Calibration standard source	Primer (forward and reverse), Sequence & Reference		BSA (3 %)
16S rRNA gene V5-V6 Bacteria	45 s/94 °C, 45 s/58 °C, 45 s/72 °C	40	*Pseudomonas putida*	FP 16S rDNA GGTAGTCYAYGCM-STAAACG (Bach *et al.*, 2002)	0.5	0.5
				RP 16S rDNA GACARCCATGCASCACCTG (Bach *et al.*, 2002)	0.5	
16S rRNA gene V2-V5 Archaea	20 s/95 °C, 60 s/55 °C (*), 60 s/72 °C (*) Touchdown -1°C per cycle	5	*Methano-baterium sp.*	SAf (i) CTAYGGGGCGCAGCAGG (Nicol *et al.*, 2003)	0.5	0.5
	20 s/95 °C, 60 s/50 °C, 60 s/72 °C	40		958r YCCGGCGTTGAMTCCAATT (Bano *et al.*, 2003)	0.5	
ITS 1 & 2 Fungi	30 s/94 °C, 30 s/50 °C, 30 s/72 °C	40	*Trichoderma viride*	ITS1 TCCGTAGGTGAACCTGCGG (White *et al.*, 1990)	1	0
				ITS4 TCCTCCGCTTATTGA-TATGC (White *et al.*, 1990)	1	

PCR reactions contained 12.5 µl of Power SybrGreen Master Mix and 2 µl of template DNA plus F- and R-primer (10 µM) and 3 % of bovine serum albumin (BSA) as given in the table (in µl).

Table S2 Primer pairs and sequences, PCR Mastermix and reaction conditions for Amplicon Sequencing.

Gene	Primer (forward and reverse), Sequence & Reference	Mastermix	V [µl]	No. of Cycles	T [°C]	t
16s rRNA Bacteria & Archaea	Arch0519 F CAGCMGCCGCGGTAA (Klindworth *et al.*, 2013)	NebNext High Fidelity Mix	12.5	1	98	5 min
		F- & R primer	0.5		98	20 sec
	Pro 805 R GACTACNVGGGTATCTAATCC (Herlemann *et al.*, 2011)	BSA (3%)	1	25	51	20 sec
		DEPC water	9.5		72	30 sec
				1	72	5 min
ITS Fungi	ITS 3 mix CATCGATGAAGAACGCAG CAACGATGAAGAACGCAG CACCGATGAAGAACGCAG CATCGATGAAGAACGTAG CATCGATGAAGAACGTGG (Tedersoo *et al.*, 2015)	NebNext High Fidelity Mix	10	1	95	15 min
		F- & R primer	0.5		95	30 sec
		BSA (3%)	0	27	55	30 sec
		DEPC water	13		72	60 sec
				1	72	10 min
	ITS 4 mix TCCTCCGCTTATTGATATGC TCCTGCGCTTATTGATATGC TCCTCGCCTTATTGATATGC TCCTCCGCTGAWTAATATGC (Tedersoo *et al.*, 2015)					

PCR reactions contained 1 µl (3 ng μl^{-1}) of template DNA plus NebtNext Master Mix, F- and R-primer (10 µM), 3 % of bovine serum albumin (BSA) and Diethylpyrocarbonate (DEPC) treated water as given in the table (in µl).

Table S3 Abiotic soil properties of nitrogen (ammonium, nitrate and dissolved organic nitrogen (DON)) and carbon (dissolved organic (DOC) and inorganic (DIC) carbon) pools, chlorophyll *a* (µg per g soil dry weight (dw)), and pH are given as mean values and standard deviation. Treatments are given for tillage (conventional (cT) vs. reduced tillage (rT)), organic treatment (without (-org) vs. with crop residues (+org)), and mineral fertilization amount (120 vs. 240 kg N/ha·a).

Compartment		Ammonium		Nitrate		DON		DOC		DIC		Chlorophyll *a*		pH	
	Treatment	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd	mean	sd
bare soil	pT 120-org	0.52	0.09	7.61	1.90	1.90	0.35	23.25	25.33	5.41	5.06	3.07	1.78	6.14	0.12
bare soil	pT 240 -org	3.02	4.29	17.26	8.84	7.51	8.43	25.51	27.98	8.13	3.86	2.80	0.84	5.90	0.18
bare soil	pT 120+org	0.59	0.17	11.72	0.56	2.26	0.48	8.81	1.91	13.03	4.83	4.63	1.36	6.15	0.07
bare soil	pT 240 +org	0.64	0.04	17.82	3.95	4.02	0.79	14.56	3.48	11.13	4.20	3.85	3.22	6.43	0.06
bare soil	rT 120-org	0.59	0.05	9.61	2.40	1.75	0.32	12.39	1.90	4.24	1.40	3.40	1.69	6.05	0.05
bare soil	rT 240 -org	0.70	0.13	23.22	6.10	4.65	1.67	14.12	1.08	2.64	0.68	3.46	0.70	5.92	0.22
bare soil	rT 120+org	0.58	0.10	16.40	0.82	2.89	1.31	15.36	4.83	3.06	0.93	2.93	0.76	6.20	0.21
bare soil	rT 240 +org	0.54	0.03	21.52	6.15	3.57	0.58	14.54	3.18	5.49	3.84	2.80	0.62	6.12	0.14
biocrust	pT 120-org	1.40	1.26	28.14	17.15	5.73	2.56	18.09	6.73	25.15	17.28	23.28	9.42	6.87	0.11
biocrust	pT 240 -org	2.92	3.68	39.65	9.96	5.09	2.49	19.82	4.82	13.79	5.30	23.16	7.53	6.83	0.10
biocrust	pT 120+org	0.53	0.04	25.55	13.70	4.56	2.24	15.70	5.56	11.32	5.49	17.37	4.60	6.70	0.16
biocrust	pT 240 +org	0.61	0.17	44.95	4.09	7.71	0.92	18.53	3.41	15.78	8.90	25.18	14.58	6.81	0.12
biocrust	rT 120-org	0.70	0.07	31.45	11.98	5.22	2.43	15.87	4.58	8.57	2.50	18.81	8.68	6.62	0.06
biocrust	rT 240 -org	0.69	0.05	37.55	18.57	6.69	5.31	20.40	6.13	10.53	10.02	22.69	15.26	6.68	0.16
biocrust	rT 120+org	0.54	0.13	31.98	10.34	6.27	2.83	16.63	1.92	5.65	1.78	10.95	3.59	6.48	0.03
biocrust	rT 240 +org	0.63	0.31	55.04	16.16	10.87	5.35	29.39	5.26	9.49	5.33	25.32	5.96	6.75	0.17

Table S4 DNA concentration [ng µg-1] as mean ± standard deviation of replicates (n = 3) per treatment, including extraction blanks. Treatments are given for tillage (conventional (cT) vs. reduced tillage (rT)), organic amendments (without (-org) vs. with crop residues (+org)), and mineral fertilization amounts (120 vs. 240 kg N/ha·a).

Compartment		Sample Name	DNA concentration [ng μg^{-1}]
bare soil	cT 120 -org	31,00 ± 11,11	
	cT 240 -org	16,63 ± 8,99	
	cT 120 +org	27,95 ± 3,71	
	cT 240 +org	31,47 ± 18,76	
	rT 120 -org	31,94 ± 4,37	
	rT 240 -org	27,26 ± 1,49	
	rT 120 +org	47,50 ± 4,26	mean of all bare soils
	rT 240 +org	37,73 ± 18,43	31.44
biocrust	cT 120 -org	58,67 ± 4,07	
	cT 240 -org	50,01 ± 16,26	
	cT 120 +org	47,84 ± 11,18	
	cT 240 +org	40,92 ± 7,74	
	rT 120 -org	49,99 ± 14,37	
	rT 240 -org	48,07 ± 6,49	
	rT 120 +org	42,52 ± 10,21	mean of all biocrusts
	rT 240 +org	55,05 ± 6,94	49.13
	extraction blank	0,95 ± 0,77	

Table S5 Copies of target region (per gram soil dry weight (dw)) 16S rRNA gene for Archaea and Bacteria and ITS region for Fungi are given as mean values and standard deviation. Treatments are given for tillage (conventional (cT) vs. reduced tillage (rT)), organic treatment (without (-org) vs. with crop residues (+org)), and mineral fertilization amount (120 vs. 240 kg N/ha·a).

Compartment	Treatment	16S rRNA gene Archaea		16S rRNA gene Bacteria		ITS Fungi	
		mean	sd	mean	sd	mean	sd
bare soil	pT 120-org	30,158,522	28,786,476	15,833,228,596	9,785,044,739	1,209,683,879	501,324,052
bare soil	pT 240 -org	1,115,774	958,086	3,912,995,601	2,268,358,142	13,841,559	7,319,566
bare soil	pT 120+org	4,395,344	2,490,443	6,692,403,534	1,283,100,670	81,588,451	33,006,787
bare soil	pT 240 +org	1,757,812	1,135,305	7,234,086,396	4,298,489,586	678,731,830	585,252,493
bare soil	rT 120-org	877,913	543,421	6,270,253,214	2,329,444,537	9,924,290	6,979,023
bare soil	rT 240 -org	1,055,357	1,273,463	8,518,275,274	4,108,343,309	142,637,751	210,868,316
bare soil	rT 120+org	19,603,592	21,890,057	28,592,677,780	8,498,428,141	1,224,060,890	1,958,259,576
bare soil	rT 240 +org	3,697,594	2,673,294	9,776,191,756	1,245,978,495	1,181,556,224	1,080,255,831
biocrust	pT 120-org	19,479,474	14,160,382	44,179,890,625	8,560,733,258	4,746,620,424	1,326,688,963
biocrust	pT 240 -org	13,931,087	18,283,691	51,267,935,619	34,587,018,705	5,813,208,249	1,442,820,267
biocrust	pT 120+org	22,050,121	20,498,002	26,049,059,757	6,431,184,875	2,035,111,867	372,517,387
biocrust	pT 240 +org	8,196,163	5,786,396	31,974,123,768	7,124,875,252	3,961,607,216	2,250,027,957
biocrust	rT 120-org	18,347,028	4,292,938	32,742,741,321	8,817,402,780	3,675,043,988	372,941,058
biocrust	rT 240 -org	3,866,121	2,928,979	33,264,921,660	11,599,851,124	4,966,968,679	2,752,185,396
biocrust	rT 120+org	22,638,979	13,678,676	24,441,080,206	13,490,605,040	4,708,856,924	4,918,161,917
biocrust	rT 240 +org	4,272,187	3,072,679	37,129,254,298	9,652,908,098	3,960,744,621	2,234,973,563

Table S6 16S rRNA reads for bacterial/archaeal sequencing through pipeline analysis from raw files (fastq) to taxonomy assignment.

			Raw data	Quality filtering						Taxonomy Assignment						
Compart-ment	Sample Name	Repli-cate	Fastq	After Adapter-removal	After Quality Filtering	After Denoising	After Denoising Merged	Without Chimeras	reads lost [%]	16S Without Kingdom Unspec.	Bacteria & Archaea	Chloro-plasts [%]	Mitochon-dria [%]	Bacteria	Bacteria (Phylum unspec.) [%]	Archaea [%]
bare soil	cT 120 -org	a	58985	58959	57279	55266	48976	48871	17.1	48675	48626	5.8	0.2	45460	0.5	0.5
bare soil	cT 120 -org	b	57567	57470	54922	52406	44923	44521	22.7	44435	44423	4.3	0.7	41493	0.2	1.7
bare soil	cT 120 -org	c	50577	50543	49026	47177	42330	42266	16.4	42233	42222	4.7	0.6	39803	0.3	0.4
bare soil	cT 240 -org	a	53140	53087	51312	50047	46563	46287	12.9	45881	45672	25.4	0.5	33788	1.0	0.0
bare soil	cT 240 -org	b	67563	67514	65936	63798	57041	55876	17.3	56697	56661	8.0	0.7	51530	0.1	0.4
bare soil	cT 240 -org	c	113199	113102	109960	107138	98380	97281	14.1	98035	97908	5.6	0.3	91891	0.3	0.2
bare soil	cT 120 +org	a	53549	53484	51790	49709.5	43897	43871	18.1	43729	43611	8.9	0.5	39474	0.5	0.2
bare soil	cT 120 +org	b	65701	65657	63474	60741	52385	52296	20.4	52175	52161	4.0	0.3	49273	0.2	1.4
bare soil	cT 120 +org	c	62575	62520	60556	57849	48920	48663	22.2	48632	48559	7.7	0.3	44358	0.7	0.7
bare soil	cT 240 +org	a	70473	70362	67982	65852	58905	58704	16.7	58611	58559	6.3	0.2	54694	0.4	0.1
bare soil	cT 240 +org	b	87981	87936	85382	82744	73603	72765	17.3	72817	72770	5.4	0.3	68519	0.2	0.2
bare soil	cT 240 +org	c	64374	64213	62301	59489	50226	50111	22.2	50023	50003	3.4	0.3	47658	0.1	1.0
bare soil	rT 120 -org	a	98663	98519	95095	92360	82864	82291	16.6	81903	81732	12.6	0.6	70633	0.7	0.5
bare soil	rT 120 -org	b	78409	78294	75914	73582	66158	65978	15.9	65715	65662	7.4	0.4	60351	0.3	0.3
bare soil	rT 120 -org	c	41321	41265	40064	38078	32143	32067	22.4	31963	31955	6.5	0.3	29654	0.4	0.5
bare soil	rT 240 -org	a	267150	266923	259562	255710	241211	234062	12.4	238655	238353	9.4	0.6	214327	0.6	0.1
bare soil	rT 240 -org	b	50560	50520	47479	45229	35467	35467	29.9	35286	35269	11.0	0.8	31084	0.6	0.0
bare soil	rT 240 -org	c	72707	72536	70204	67999	60864	60661	16.6	60538	60499	9.4	0.5	54449	0.5	0.1
bare soil	rT 120 +org	a	82287	82284	60984	54654	27901	27668	66.4	27555	27543	8.4	0.5	25016	0.2	0.3
bare soil	rT 120 +org	b	77634	77520	74846	71908.5	62047	61862	20.3	61702	61638	10.0	0.5	54727	0.7	0.9

bare soil	rT 120 +org	c	73037	72978	70897	67920	57595	57385	21.4	57200	57027	8.9	0.4	51308	0.5	0.9
bare soil	rT 240 +org	a	62427	62342	60526	58011	50536	50272	19.5	50218	50141	3.6	0.4	48047	0.2	0.2
bare soil	rT 240 +org	b	78846	78800	75913	73191	62843	62615	20.6	62300	62158	13.2	1.0	53189	0.6	0.3
bare soil	rT 240 +org	c	66239	66148	64391	61870	53826	53423	19.3	53192	53018	10.2	0.5	47149	0.6	0.4
biocrust	cT 120 -org	a	71163	71122	68267	66175	58429	58150	18.3	57995	57852	7.2	0.5	53279	0.4	0.1
biocrust	cT 120 -org	b	75375	75315	73053	70388	62857	62451	17.1	62327	62284	5.5	0.3	58327	0.3	0.6
biocrust	cT 120 -org	c	54951	54908	53189	51435.5	47011	46938	14.6	46821	46796	6.4	0.2	43598	0.5	0.2
biocrust	cT 240 -org	a	57736	57690	55433	53991	48829	48578	15.9	48278	48189	10.9	0.0	42881	0.6	0.0
biocrust	cT 240 -org	b	49763	49710	48149	45906	39937	39848	19.9	39775	39737	8.2	0.2	36202	0.4	0.6
biocrust	cT 240 -org	c	46869	46796	45317	43597	39166	39112	16.6	38934	38873	13.0	0.5	33626	0.4	0.1
biocrust	cT 120 +org	a	47105	47068	45809	43895	38748	38337	18.6	38227	38119	13.4	1.0	32571	0.4	0.2
biocrust	cT 120 +org	b	62340	62278	60542	58267	51669	51606	17.2	51520	51502	10.7	0.5	45310	0.2	1.0
biocrust	cT 120 +org	c	52723	52620	50879	48744	42488	42264	19.8	42159	42030	10.4	0.4	37364	1.8	0.3
biocrust	cT 240 +org	a	203856	203799	198520	194518	181574	178235	12.6	180265	179930	8.9	0.5	162670	0.5	0.2
biocrust	cT 240 +org	b	49626	49588	47940	46006	40390	40234	18.9	40116	40066	6.9	0.2	37184	0.3	0.1
biocrust	cT 240 +org	c	79731	79687	77058	74709	67621	67227	15.7	67142	67074	5.7	0.1	63016	0.3	0.3
biocrust	rT 120 -org	a	76639	76448	73304	70894	64226	63963	16.5	63653	63478	15.3	1.2	52700	1.4	0.6
biocrust	rT 120 -org	b	60751	60679	58669	56624	51023	50937	16.2	50650	50562	17.8	1.4	40834	1.1	0.0
biocrust	rT 120 -org	c	71298	71210	69049	66835	61002	60909	14.6	60561	60512	21.8	0.4	46812	1.8	0.5
biocrust	rT 240 -org	a	63397	63319	61068	59617	55614	55614	12.3	55232	55099	28.3	1.2	38799	1.7	0.0
biocrust	rT 240 -org	b	50143	50081	48803	47177	42769	42712	14.8	42287	42217	19.4	0.9	33650	0.5	0.0
biocrust	rT 240 -org	c	76220	76178	74209	71638	63157	62894	17.5	62450	62425	14.7	0.2	52979	0.3	0.3
biocrust	rT 120 +org	a	245145	244978	238278	232951	213831	208235	15.1	211255	210921	10.7	0.3	186111	0.3	0.8
biocrust	rT 120 +org	b	57148	57063	55158	52389	43661	43520	23.8	43303	43244	10.8	0.3	38099	0.3	0.9
biocrust	rT 120 +org	c	52033	51926	50178	47804	41101	40954	21.3	40815	40693	14.2	0.8	34429	0.5	0.5

biocrust	rT 240 +org	a	41050	41016	39631	38096	34055	33953	17.3	33411	33288	37.2	1.5	20405	1.7	0.0
biocrust	rT 240 +org	b	51582	51537	49737	47809	42866	42816	17.0	42384	42231	37.8	0.8	25904	1.7	0.1
biocrust	rT 240 +org	c	50008	49958	48107	46465	41800	41700	16.6	41509	41363	15.3	0.2	34949	0.8	0.1
	extraction blank		167	122	46	1.5	0	0	100.0	0	0	0.0	0.0	0	0.0	0.0
	extraction blank		173	97	44	6	5	5	97.1	5	5	0.0	0.0	5	0.0	0.0
	extraction blank		319	197	174	75.5	64	64	79.9	64	64	9.4	0.0	58	0.0	0.0
	extraction blank		104	81	68	35	35	35	66.3	35	35	0.0	0.0	35	0.0	0.0
	PCR NTC		106	18	9	1	0	0	100.0	0	0	0.0	0.0	0	0.0	0.0
	PCR NTC		107	68	8	1	0	0	100.0	0	0	0.0	0.0	0	0.0	0.0
	PCR NTC		184	169	15	1.5	0	0	100.0	0	0	0.0	0.0	0	0.0	0.0
	PCR NTC		111	49	12	1	0	0	100.0	0	0	0.0	0.0	0	0.0	0.0

Table S7 ITS reads for fungal sequencing through pipeline analysis from raw files (fastq) to taxonomy assignment.

			Raw data	Quality filtering						Taxonomy Assignment		
Compartment	Sample Name	Repli-cate	Fastq	After Adapter-removal	After Quality Filtering	After Denoising	After Denoising Merged	Without Chimeras	reads lost [%]	ITS Without Kingdom Unspec.	Fungi	Fungi (Phylum unspec.) [%]
bare soil	cT 120 -org	a	110253	108159	95108	93742	88926	77820	28.1	77754	75005	39.2
bare soil	cT 120 -org	b	237638	232879	203872	201766	194726	164838	29.2	159177	150984	40.7
bare soil	cT 120 -org	c	107262	106297	92048	90505	81502	71115	33.1	70154	66132	46.1
bare soil	cT 240 -org	a	256417	255102	232995	231861	227239	191363	25.0	191358	186141	31.3
bare soil	cT 240 -org	b	280742	275427	239576	237741	228054	189210	31.3	183131	175637	57.4
bare soil	cT 240 -org	c	167366	164228	143771	142153	136093	111948	31.8	110747	108171	57.2
bare soil	cT 120 +org	a	146038	144576	124040	122535	113998	96907	33.0	93668	91745	56.9
bare soil	cT 120 +org	b	203931	201300	175342	173449	164695	139215	30.8	127546	121696	43.1
bare soil	cT 120 +org	c	190005	187025	165575	163551	152907	130693	30.1	128283	124342	29.6
bare soil	cT 240 +org	a	72451	72065	63941	62976	60751	53511	25.7	54049	52831	29.3
bare soil	cT 240 +org	b	113833	113063	101810	100412	96401	82782	26.8	82125	75092	34.2
bare soil	cT 240 +org	c	170995	168851	147455	145914	138057	111873	33.7	114080	96514	22.7
bare soil	rT 120 -org	a	92166	90433	80481	79264	75006	64817	28.3	64794	62889	55.8
bare soil	rT 120 -org	b	62790	62293	55755	54533	51945	46979	24.6	45588	44777	26.3
bare soil	rT 120 -org	c	82956	82376	72732	71433	67588	59360	27.9	59279	57675	46.5
bare soil	rT 240 -org	a	101438	100716	91361	90354	86793	73506	27.0	73502	65553	35.9
bare soil	rT 240 -org	b	158627	157801	146359	144584	137487	120147	23.9	121010	117387	44.4
bare soil	rT 240 -org	c	80779	80385	71559	70367	67857	58978	26.6	58978	57400	45.5
bare soil	rT 120 +org	a	76991	76476	67502	66144	61959	56049	26.7	55856	54744	22.4
bare soil	rT 120 +org	b	62542	61619	55033	53676	50083	44511	27.8	44223	42183	42.9

bare soil	rT 120 +org	c	55525	55064	49323	48277	45342	39765	27.8	39751	38610	54.3
bare soil	rT 240 +org	a	43844	43527	39502	38482	35622	32364	25.6	32362	30686	31.1
bare soil	rT 240 +org	b	51264	50929	45191	44197	41823	37359	26.6	37359	36839	41.8
bare soil	rT 240 +org	c	51907	51350	46252	45160	42760	37844	26.3	37989	37642	33.7
biocrust	cT 120 -org	a	49739	49464	44189	43356	41336	36433	26.3	36376	35973	38.4
biocrust	cT 120 -org	b	33610	33417	29481	28685	27223	24303	27.3	23979	23892	57.9
biocrust	cT 120 -org	c	45401	45246	38134	37133	34815	31580	30.2	31840	31481	40.6
biocrust	cT 240 -org	a	47948	47279	42551	41973	40898	33386	29.4	33386	30256	38.0
biocrust	cT 240 -org	b	61516	61100	54907	54184	52434	45349	25.8	45235	43314	36.1
biocrust	cT 240 -org	c	47958	47585	42347	41419	38843	34107	28.3	34019	33433	59.0
biocrust	cT 120 +org	a	49041	48858	41805	40994	35078	30005	38.6	29558	29311	82.0
biocrust	cT 120 +org	b	99630	99229	85276	83886	75229	66558	32.9	62665	61405	59.6
biocrust	cT 120 +org	c	46588	46248	38186	37052	33730	30940	33.1	30517	30081	49.8
biocrust	cT 240 +org	a	106824	105597	94348	93058	83625	70841	32.9	70440	68895	63.6
biocrust	cT 240 +org	b	84844	84524	75943	74809	67494	58494	30.8	57341	56136	75.8
biocrust	cT 240 +org	c	115663	115126	104071	103051	97807	81247	29.4	81206	80219	61.0
biocrust	rT 120 -org	a	99687	98666	87695	86414	81565	68238	30.8	68191	66480	67.0
biocrust	rT 120 -org	b	119963	119059	107335	105693	98848	84457	29.1	83919	81789	50.7
biocrust	rT 120 -org	c	125528	124016	108144	106490	101462	84987	31.5	84231	82217	44.5
biocrust	rT 240 -org	a	94283	93544	83589	82586	77846	65398	30.1	65306	61718	68.9
biocrust	rT 240 -org	b	85443	84845	75636	74555	69023	59764	29.6	59678	57535	57.0
biocrust	rT 240 -org	c	107944	107527	95580	94201	89999	76848	28.5	78275	77490	29.1
biocrust	rT 120 +org	a	100747	100358	90455	89258	83397	73332	26.9	72204	70179	47.5
biocrust	rT 120 +org	b	99699	99387	89306	88014	80753	70532	29.0	70506	69221	61.3
biocrust	rT 120 +org	c	101200	100037	89406	88049	83932	69380	30.6	69370	68135	48.4

biocrust	rT 240 +org	a	92574	88405	79214	78074	73846	58867	33.4	58854	57389	77.2
biocrust	rT 240 +org	b	103593	102297	89346	87760	81417	64663	36.8	64613	63258	72.5
biocrust	rT 240 +org	c	98446	95343	84287	83145	78571	63383	33.5	63537	62312	53.8
	extraction blank		9425	9390	1612	1582	1579	1253	86.7	1253	1253	0.0
	extraction blank		5075	4990	53	16	11	11	99.8	11	11	36.4
	extraction blank		135	133	15	1	0	0	100.0	0	0	0.0
	extraction blank		14470	13918	45	17	6	6	100.0	6	6	100.0
	PCR NTC		7558	7260	27	3	3	3	100.0	3	3	100.0
	PCR NTC		17833	1424	5	2	2	2	99.9	2	2	0.0
	PCR NTC		3795	3143	19	5	3	3	99.9	3	3	100.0
	PCR NTC		8201	5487	21	4	0	0	100.0	0	0	0.0

Table S8 Alpha diversity for prokaryotic 16S rRNA and ITS Fungi for Shannon diversity (S), richness (R) and Pielou's eveness (P) as mean ± standard deviation. Treatments are given for tillage (conventional (cT) vs. reduced tillage (rT)), organic amendments (without (-org) vs. with crop residues (+org)), and mineral fertilization amounts (120 vs. 240 kg N/ha·a)

Gene	Compartment	Tillage	Mineral Fertilization Amounts	Organic Amendments	S	R	P
Prokaryotic 16S rRNA	bare soil	cT	120	-org	5.90 ± 0.46	890 ± 118.1	0.869 ± 0.053
				+org	6.02 ± 0.43	1003 ± 170.7	0.872 ± 0.041
			240	-org	5.92 ± 0.13	1038 ± 383.0	0.859 ± 0.03
				+org	6.32 ± 0.15	1138 ± 72.5	0.898 ± 0.018
		rT	120	-org	6.02 ± 0.38	1037.7 ± 306.1	0.871 ± 0.018
				+org	6.09 ± 0.87	946.3 ± 628.2	0.926 ± 0.015
			240	-org	6.32 ± 0.48	1314.7 ± 795.2	0.898 ± 0.017
				+org	6.48 ± 0.06	1153.3 ± 34.5	0.919 ± 0.005
	biocrust	cT	120	-org	5.93 ± 0.31	928.7 ± 168	0.869 ± 0.023
				+org	5.81 ± 0.35	820.3 ± 150.9	0.866 ± 0.032
			240	-org	5.93 ± 0.02	797.3 ± 42.1	0.888 ± 0.008
				+org	6.13 ± 0.42	1245.7 ± 593.1	0.868 ± 0.007
		rT	120	-org	6.05 ± 0.03	965.7 ± 46.7	0.88 ± 0.009
				+org	6.48 ± 0.36	1447.7 ± 845.4	0.903 ± 0.017
			240	-org	6.11 ± 0.36	939 ± 287.9	0.896 ± 0.015
				+org	5.91 ± 0.01	677 ± 69.8	0.908 ± 0.016
ITS Fungi	bare soil	cT	120	-org	4.66 ± 0.25	512.3 ± 107.9	0.748 ± 0.028
				+org	4.80 ± 0.27	537 ± 96.4	0.764 ± 0.029

		240	-org	3.93 ± 0.92	438.7 ± 137.3	0.645 ± 0.119
			+org	3.98 ± 0.30	327.7 ± 86.4	0.691 ± 0.049
	rT	120	-org	4.28 ± 0.24	339.7 ± 23.1	0.735 ± 0.038
			+org	4.50 ± 0.08	332.7 ± 41.7	0.776 ± 0.008
		240	-org	4.22 ± 0.33	332.7 ± 74	0.727 ± 0.03
			+org	4.44 ± 0.08	303.7 ± 7.8	0.776 ± 0.011
biocrust	cT	120	-org	3.71 ± 0.23	167.7 ± 18.9	0.725 ± 0.042
			+org	3.98 ± 0.26	238.7 ± 93.9	0.733 ± 0.003
		240	-org	3.52 ± 0.53	162.7 ± 48.4	0.694 ± 0.065
			+org	4.02 ± 0.29	302.7 ± 55.2	0.705 ± 0.030
	rT	120	-org	4.33 ± 0.19	387.7 ± 47	0.728 ± 0.017
			+org	4.17 ± 0.25	345.3 ± 14.8	0.713 ± 0.038
		240	-org	4.11 ± 0.28	303.7 ± 94.5	0.722 ± 0.019
			+org	3.94 ± 0.30	286 ± 24.9	0.696 ± 0.044

Table S9 p and F values of linear models for alpha diversity of 16S rRNA Bacteria/Archaea and ITS Fungi as Shannon diversity (S), richness (R) and Pielou's eveness (P). Significant values ($p \leq 0.05$) are marked in bold.

Gene	Variable	Biocrust effect		Com-partment	Tillage		Mineral Fertilizer Amount		Organic Amend-ments		Tillage * Organic Amend-ments		Tillage * Mineral Fertilizer Amount		Mineral Fer-tilizer Amount * Organic Amend-ments		Tillage * Mineral Fer-tilizer Amount * Organic Amend-ments	
		F	p		F	p	F	p	F	p	F	p	F	p	F	p	F	p
Prokaryotic 16S rRNA	S	0.60	0.441	bare soil	0.90	0.356	0.92	0.351	1.94	0.182	0.19	0.666	0.26	0.617	0.30	0.590	0.04	0.846
				biocrust	2.60	0.126	0.35	0.565	0.12	0.736	0.13	0.727	3.18	0.094	0.39	0.543	3.87	0.067
	R	0.45	0.506	bare soil	0.00	0.974	0.01	0.929	1.19	0.291	0.56	0.466	0.21	0.656	0.22	0.649	0.16	0.694
				biocrust	0.06	0.805	0.29	0.599	1.13	0.303	0.29	0.600	3.94	0.065	0.05	0.830	5.54	**0.032**
	P	0.21	0.647	bare soil	6.02	**0.026**	6.10	**0.025**	0.60	0.451	0.48	0.497	0.00	0.949	0.00	0.968	2.24	0.154
				biocrust	10.63	**0.005**	0.17	0.688	2.02	0.174	3.94	0.065	0.01	0.941	1.03	0.326	0.05	0.820
ITS Fungi	S	8.82	**0.005**	bare soil	0.10	0.755	0.96	0.342	5.86	**0.028**	0.06	0.804	4.20	0.057	0.00	0.983	0.00	0.993
				biocrust	6.66	**0.020**	0.90	0.357	1.44	0.248	4.72	**0.045**	0.23	0.635	0.24	0.632	0.29	0.599
	R	16.33	**0.000**	bare soil	11.94	**0.003**	0.94	0.346	5.65	**0.030**	0.14	0.709	2.78	0.115	1.27	0.276	0.68	0.422
				biocrust	24.64	**0.000**	4.77	**0.044**	0.44	0.516	8.77	**0.009**	3.32	0.087	1.24	0.281	0.47	0.501
	P	1.08	0.304	bare soil	3.57	0.077	2.86	0.110	4.34	0.054	0.03	0.876	3.72	0.072	0.27	0.613	0.14	0.716
				biocrust	0.01	0.940	0.11	0.749	1.85	0.193	1.04	0.323	0.38	0.545	0.02	0.888	0.06	0.803

Table S10 p and F values of highly abundant bacterial families (at least 2% per replicate) reacting on interacting management effects. Families shown in bold are affected in bare soil and biocrust. Significant effects ($p < 005$) are shown in bold.

Compartment	Taxa	Tillage		Mineral Fertilizer Amount		Organic Amendments		Tillage * Organic Amendments		Tillage * Mineral Fertilizer Amount		Mineral Fertilizer Amount * Organic Amendments		Tillage * Mineral Fertilizer Amount * Organic Amendments	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p
bare soil	**k__Bacteria, p__Bacteroidetes, c__Bacteroidia, o__Sphingobacteriales, f__env.OPS_17**	0.27	0.608	4.41	0.052	1.06	0.318	0.37	0.552	0.66	0.430	3.29	0.088	5.96	**0.027**
	k__Bacteria, p__Cyanobacteria, c__Oxyphotobacteria, o__Leptolyngbyales, f__Leptolyngbyaceae	0.57	0.463	1.58	0.226	1.65	0.217	6.10	**0.025**	0.08	0.785	0.08	0.780	0.35	0.562
	k__Bacteria, p__Proteobacteria, c__Gammaproteobacteria, o__Alteromonadales, f__Alteromonadaceae	1.60	0.223	1.16	0.297	4.85	**0.043**	4.74	**0.045**	2.04	0.172	1.54	0.232	4.05	0.061
	k__Bacteria, p__Proteobacteria, c__Gammaproteobacteria, o__Xanthomonadales, f__Xanthomonadaceae	0.34	0.569	37.13	**0.000**	26.29	**0.000**	0.40	0.535	11.87	**0.003**	0.84	0.373	6.20	**0.024**
	k__Bacteria, p__Verrucomicrobia, c__Verrucomicrobiae, o__Chthoniobacterales, f__Chthoniobacteraceae	5.40	**0.034**	3.86	0.067	7.23	**0.016**	0.70	0.416	0.37	0.552	0.50	0.492	4.87	**0.042**
biocrust	k__Bacteria, p__Actinobacteria, c__Actinobacteria, o__Propionibacteriales, f__Nocardioidaceae	5.62	**0.031**	4.47	0.051	5.91	**0.027**	0.86	0.367	7.08	**0.017**	0.26	0.615	7.12	**0.017**
	k__Bacteria, p__Actinobacteria, c__Actinobacteria, o__Pseudonocardiales, f__Pseudonocardiaceae	2.96	0.105	5.10	0.038	5.89	**0.027**	0.32	0.580	13.98	**0.002**	0.34	0.565	12.02	**0.003**
	k__Bacteria, p__Bacteroidetes, c__Bacteroidia, o__Chitinophagales, f__Chitinophagaceae	23.03	**0.000**	0.27	0.609	6.75	**0.019**	2.54	0.130	0.90	0.356	5.42	**0.033**	6.59	**0.021**
	k__Bacteria, p__Bacteroidetes, c__Bacteroidia, o__Sphingobacteriales, f__env.OPS_17	7.81	**0.013**	0.76	0.395	2.40	0.141	**0.03**	0.872	4.98	**0.040**	0.53	0.476	6.45	**0.022**
	k__Bacteria, p__Bacteroidetes, c__Bacteroidia, o__Sphingobacteriales, f__Sphingobacteriaceae	13.54	**0.002**	**0.01**	0.908	8.62	**0.010**	5.17	**0.037**	3.24	0.091	1.83	0.195	3.41	0.083
	k__Bacteria, p__Cyanobacteria, c__Oxyphotobacteria, o__Oxyphotobacteria_Incertae_Sedis, f__Unknown_Family	7.95	**0.012**	3.97	0.064	1.65	0.218	5.65	**0.030**	0.75	0.400	2.55	0.130	0.43	0.521
	k__Bacteria, p__Gemmatimonadetes, c__Longimicrobia, o__Longimicrobiales, f__Longimicrobiaceae	3.82	0.068	0.06	0.811	**0.00**	0.970	4.44	0.051	5.96	**0.027**	0.64	0.435	2.74	0.118
	k__Bacteria, p__Proteobacteria, c__Alphaproteobacteria, o__Rhizobiales, f__Beijerinckiaceae	20.84	**0.000**	0.22	0.644	8.63	**0.010**	0.05	0.821	4.56	**0.048**	0.01	0.919	5.34	**0.035**
	k__Bacteria, p__Proteobacteria, c__Deltaproteobacteria, o__Myxococcales, f__Archangiaceae	12.72	**0.003**	0.50	0.490	7.71	**0.013**	2.43	0.139	8.44	**0.010**	4.47	0.051	17.38	**0.001**
	k__Bacteria, p__Verrucomicrobia, c__Verrucomicrobiae, o__Chthoniobacterales, f__Chthoniobacteraceae	4.70	**0.046**	4.71	**0.045**	30.36	**0.000**	4.46	0.051	2.20	0.157	5.68	**0.030**	2.21	0.156
	k__Bacteria, p__Verrucomicrobia, c__Verrucomicrobiae, o__Verrucomicrobiales, f__Rubritaleaceae	0.54	0.475	5.14	0.038	30.33	**0.000**	2.86	0.110	12.75	**0.003**	0.06	0.805	0.17	0.685

Table S11 p and F values of highly abundant fungal families (at least 2% per replicate) reacting on interacting management effects. Families shown in bold are affected in bare soil and biocrust. Significant effects ($p < 005$) are shown in bold.

Compartment	Taxa	Tillage		Mineral Fertilizer Amount		Organic Amendments		Tillage * Organic Amendments		Tillage * Mineral Fertilizer Amount		Mineral Fertilizer Amount * Organic Amendments		Tillage * Mineral Fertilizer Amount * Organic Amendments	
		F	p	F	p	F	p	F	p	F	p	F	p	F	p
bare soil	k__Fungi, p__Ascomycota, c__Dothideomycetes, o__Pleosporales, f__Pleosporales_fam_Incertae_sedis	**0.00**	0.987	2.65	0.123	1.84	0.194	7.92	**0.012**	1.52	0.236	0.02	0.900	2.78	0.115
	k__Fungi, p__Ascomycota, c__Leotiomycetes, o__Helotiales, f__Helotiales_fam_Incertae_sedis	11.76	**0.003**	20.79	**0.000**	2.36	0.144	13.70	**0.002**	1.91	0.186	0.14	0.712	0.85	0.370
	k__Fungi, p__Ascomycota, c__Lichinomycetes, o__Lichinales, f__Lichinaceae	11.20	**0.004**	7.82	**0.013**	1.15	0.300	8.31	**0.011**	0.72	0.408	0.09	0.767	0.08	0.781
	k__Fungi, p__Ascomycota, c__Sordariomycetes, o__Sordariales, f__Lasiosphaeriaceae	5.10	**0.038**	12.27	**0.003**	9.04	**0.008**	0.13	0.718	4.88	**0.042**	1.81	0.197	0.37	0.551
	k__Fungi, p__Basidiomycota, c__Cystobasidiomycetes, o__Cystobasidiomycetes_ord_Incertae_sedis, f__Cystobasidiomycetes_fam_Incertae_sedis	5.30	**0.035**	3.35	0.086	3.80	0.069	2.23	0.155	1.65	0.217	6.01	**0.026**	5.26	**0.036**
	k__Fungi, p__Basidiomycota, c__Tremellomycetes, o__Cystofilobasidiales, f__Cystofilobasidiaceae	14.14	**0.002**	13.45	**0.002**	0.26	0.615	7.90	**0.013**	0.86	0.367	0.92	0.352	1.38	0.257
	k__Fungi, p__Basidiomycota, c__Tremellomycetes, o__Filobasidiales, f__Piskurozymaceae	5.31	**0.035**	6.27	**0.023**	5.74	**0.029**	6.89	**0.018**	0.81	0.381	0.41	0.530	0.06	0.804
	k__Fungi, p__Basidiomycota, c__Tremellomycetes, o__Tremellales, f__Tremellales_fam_Incertae_sedis	3.25	0.090	0.71	0.411	5.41	**0.034**	11.62	**0.004**	0.07	0.794	0.46	0.506	0.80	0.384
	k__Fungi, p__Zygomycota, c__Mucoromycotina_cls_Incertae_sedis, o__Mucorales, f__Mucoraceae	0.12	0.731	3.56	0.077	0.12	0.732	5.51	**0.032**	2.33	0.147	1.07	0.316	3.55	0.078
biocrust	k__Fungi, p__Ascomycota, c__Dothideomycetes, o__Pleosporales, f__Cucurbitariaceae	7.06	**0.017**	0.38	0.545	1.08	0.314	17.26	**0.001**	3.25	0.090	0.00	0.979	0.02	0.878
	k__Fungi, p__Ascomycota, c__Dothideomycetes, o__Pleosporales, f__Pleosporaceae	5.09	**0.038**	1.59	0.226	4.37	0.053	0.86	0.367	6.12	**0.025**	0.00	0.961	0.00	0.953
	k__Fungi, p__Ascomycota, c__Eurotiomycetes, o__Chaetothyriales, f__Herpotrichiellaceae	9.67	**0.007**	6.97	**0.018**	0.38	0.544	5.36	**0.034**	3.12	0.097	0.09	0.765	4.42	0.052
	k__Fungi, p__Ascomycota, c__Leotiomycetes, o__Helotiales, f__Helotiales_fam_Incertae_sedis	36.69	**0.000**	32.78	**0.000**	8.75	**0.009**	9.71	**0.007**	0.83	0.377	0.18	0.680	0.45	0.514
	k__Fungi, p__Ascomycota, c__Pezizomycotina_cls_Incertae_sedis, o__Pezizomycotina_ord_Incertae_sedis, f__Pezizomycotina_fam_Incertae_sedis	20.38	**0.000**	1.22	0.285	0.84	0.372	2.06	0.170	5.83	**0.028**	1.05	0.320	0.01	0.913
	k__Fungi, p__Ascomycota, c__Sordariomycetes, o__Hypocreomycetidae_ord_Incertae_sedis, f__Plectosphaerellaceae	2.29	0.150	3.60	0.076	0.37	0.549	1.19	0.292	5.37	**0.034**	0.50	0.492	0.44	0.518
	k__Fungi, p__Ascomycota, c__Sordariomycetes, o__Sordariales, f__Chaetomiaceae	8.24	**0.011**	**0.00**	0.961	5.64	**0.030**	6.31	**0.023**	7.66	**0.014**	0.71	0.411	0.09	0.773
	k__Fungi, p__Ascomycota, c__Sordariomycetes, o__Sordariomycetidae_ord_Incertae_sedis, f__Glomerellaceae	**0.01**	0.939	5.00	**0.040**	0.10	0.757	0.22	0.647	5.65	**0.030**	1.36	0.261	0.50	0.490
	k__Fungi, p__Basidiomycota, c__Tremellomycetes, o__Tremellales, f__Tremellales_fam_Incertae_sedis	4.85	**0.043**	24.87	**0.000**	5.02	**0.040**	2.70	0.120	0.52	0.481	10.93	**0.004**	0.43	0.521

Table S12 Top 10 network hubs as most connected families for each network separately. Network participation is given in percentage of degree of total edges and share on positive edges. Hubs not specified at the family level were ignored. Common ones in both sample types (bare soil and biocrust) are shown in bold. Missing numbers indicate that these families either do not appear in a certain network or are not below the Top 10 hubs within this network.

Compartment	Taxa			120		240		-org		+org		cT		rT	
	Phylum	Class	Family	total degree [%]	positive edges [%]	total degree [%]	positive edges [%]	total degree [%]	positive edges [%]	total degree [%]	positive edges [%]	total degree [%]	positive edges [%]	total degree [%]	positive edges [%]
Bare soil	Acido-bacteria	Acidobacteria	Acidobacteriaceae (Subgroup 1)			5.2	5.2			4.9	4.9				
		Blastocatellia (Subgroup 4)	Blastocatellaceae			8.9	1.2	9.9	8.2			6.1	3.7	8.8	6.1
	Actino-bacteria	Actinobacteria	**Intrasporangiaceae**	6.7	6.7	4.3	3.5							7.4	6.8
			Micrococcaceae	10.9	10.9							5.5	0.0		
			Nocardioidaceae	9.3	7.8	10.9	7.8	7.3	3.4	7.1	5.8			17.8	16.4
		Thermo-leophilia	**Gaiellaceae**											5.9	5.9
	Asco-mycota	Dothideomycetes	Cucurbitariaceae					8.2	6.4						
			Pleosporaceae	5.7	4.2							7.4	4.9	5.6	2.0
		Eurotiomycetes	Herpotrichiellaceae			9.2	9.2								
		Sordariomycetes	**Nectriaceae**	5.7	4.2	5.5	4.6								
			Plectos-phaerellaceae									6.1	3.7		
	Bacte-roidetes	Bacteroidia	**Chitinophagaceae**	8.3	3.1	26.2	16.7	11.6	5.6	11.2	6.9	5.5	3.7	8.1	8.1
			env.OPS_17	6.2	6.2					6.0	4.0				
	Cyano-bacteria	Oxyphoto-bacteria	Leptolyngbyaceae					6.4	4.7					5.6	4.0
			"Unknown Family"	8.8	3.1	4.3	2.9	12.5	11.6	9.4	4.5	7.1	3.1	10.2	9.9
			Devosiaceae					6.4	1.7						

			Hyphomicrobiaceae							5.8	5.1				
	Proteo-bacteria	Alpha-proteobacteria	Methyloligellaceae									8.6	8.6		
			Rhodobacteraceae									5.5	0.0		
			Sphingomonadaceae	19.2	12.4	16.4	14.9	24.9	13.3	21.0	17.2			14.6	13.3
		Gamma-proteobacteria	**Burkholderiaceae**	21.2	10.9	10.1	5.2	8.2	3.4	12.7	3.6	8.9	0.0	9.2	7.1
			Nitrosomonadaceae							6.9	4.9				
			Xanthomonadaceae					7.7	3.0						
	Verruco-microbia	Verruco-microbiae	Verruco-microbiaceae									6.1	3.7		
	Zygo-mycota	Mortierello-mycotina Class Inc. Sed.	Mortierellaceae							5.6	5.6				
	Actino-bacteria	Actinobacteria	Geodermatophilaceae	12.7	8.1	13.7	13.7							7.9	7.7
			Intrasporangiaceae									8.1	5.6		
			Kineosporiaceae							7.5	5.6				
			Micrococcaceae	5.2	5.2	6.6	6.6							6.5	6.2
			Nocardioidaceae	29.5	24.9					9.4	9.4			9.8	9.8
		Thermo-leophilia	**Gaiellaceae**	4.6	4.6										
Biocrust	Asco-mycota	Dothideomycetes	Mycosphaerellaceae					6.4	4.7						
			Pleosporaceae			9.3	8.7	11.6	11.6			11.3	6.3	6.5	4.8
			Pleosporales Family Inc. Sed.	6.9	6.9										
		Lichinomycetes	Lichinaceae							5.6	5.6				
		Sordariomycetes	**Nectriaceae**	9.8	8.1	10.4	8.7	8.7	0.0					6.5	6.5
	Bacte-roidetes	Bacteroidia	**Chitinophagaceae**	6.4	0.6	13.1	13.1			19.1	8.8			12.7	5.3
			Microscillaceae					6.4	5.8			8.8	8.8		

Basidio-mycota	Tremello-mycetes	Piskurozymaceae									7.2	7.2						
Cyano-bacteria	Oxyphoto-bacteria	"Unknown Family"	15.6	2.3									6.3	2.5				
Plancto-mycetes	Planctomycetacia	Pirellulaceae					7.0	7.0					11.3	9.4				
Proteo-bacteria	Alpha-proteobacteria	Azospirillaceae													7.4	7.2		
		Beijerinckiaceae									6.3	5.6						
		Hyphomonadaceae													5.0	5.0		
		Rhizobiaceae			7.1	2.7												
		Rhodobacteraceae					5.2	4.7	6.3	5.3	6.3	5.0						
		Sphingomonadaceae			22.4	18.6	27.3	17.4	10.6	7.2	26.9	16.3	10.8	7.2				
		Xanthobacteraceae					14.0	14.0			8.1	7.5						
	Delta-proteobacteria	Sandaracinaceae									6.3	5.0						
	Gamma-proteobacteria	**Burkholderiaceae**	11.6	11.0	9.3	8.7			7.2	5.9			12.7	10.8				
		Rhodano-bacteraceae	5.2	2.3														
		"Unknown Family"					5.8	4.7										
		Xanthomonadaceae			7.1	7.1												
Verruco-microbia	Verruco-microbiae	Chthonio-bacteraceae									6.9	5.0						
		Opitutaceae			8.7	6.6			6.6	6.6								
		Pedosphaeraceae					11.1	10.5										

2. **Figures**

Fig. S1 Pictures of Biocrusts on the fields of IOSDV LUFA Speyer. One example is shown for each treatment. October 5th, 2016.

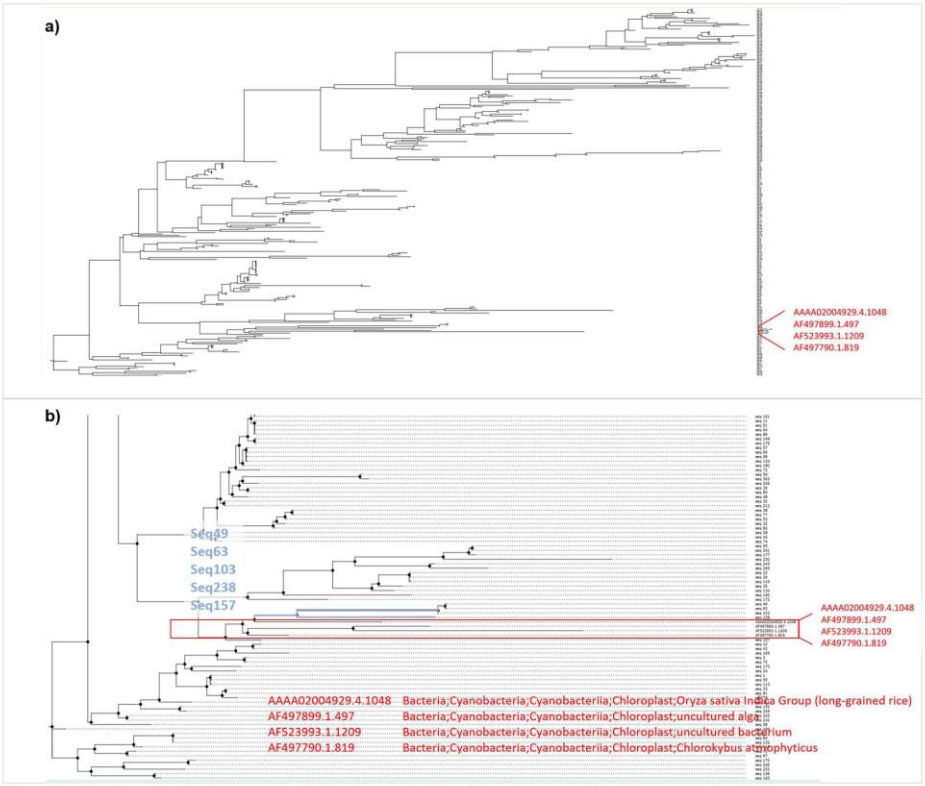

Fig. S2 Phylogenetic tree to exclude those ASVs falsely identified taxonomically as Cyanobacteria but of chloroplast origin. The tree was constructed using the "SILVA Alignment, Classification and Tree (ACT) Service" from the Silva database Version 138.1 (Quast *et al.*, 2013) including all ASVs of our study identified as Cyanobacteria and representative chloroplast reads from plants (AAAA02004929.4.1048 *Oryza sativa Indica Group* (long-grained rice)), Algae (AF497899.1.497 uncultured alga, AF497790.1.819 *Chlorokybus atmophyticus*) and Bacteria (AF523993.1.1209 uncultured bacterium). As shown in sub figure a), chloroplast reads formed a separate cluster. A zoom in sub figure b) shows that branches of five ASVs (Seq49, Seq63, Seq103, Seq238, Seq157) group close to the chloroplast reads but these ASVs display only a maximum of 1.1% of all reads (after subsampling) and were not specified further than to the order level (Bacteria; Cyanobacteria; Oxyphotobacteria; Nostocales; unspec. and Bacteria; Cyanobacteria; Oxyphotobacteria; unspec.) and were thus below the abundance cut-off for further analysis.

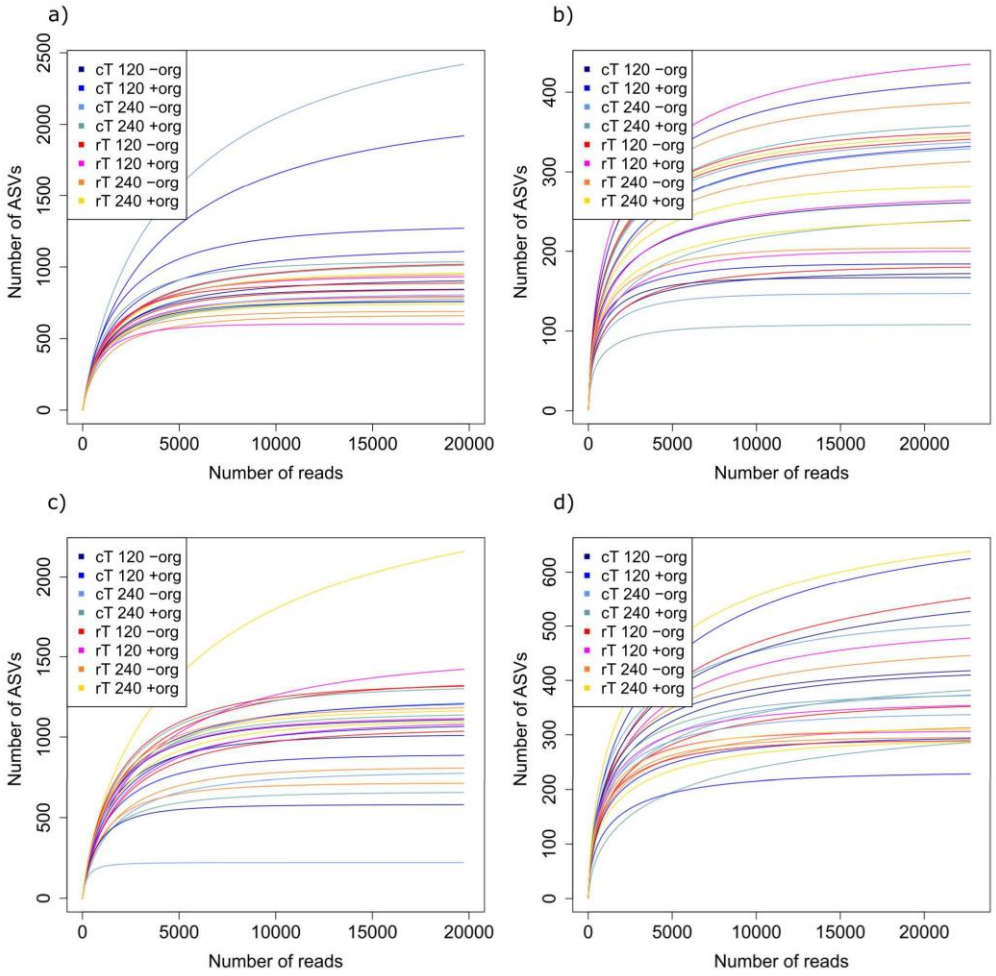

Fig. S3 Rarefaction curves on ASV level for each replicate of a) Biocrust Prokaryotes, b) Biocrust Fungi, c) Bare soil Prokaryotes, d) Bare soil Fungi on subsampled data. Treatments are given for tillage (conventional (cT) vs. reduced tillage (rT)), organic amendments (without (-org) vs. with crop residues (+org)), and mineral fertilization amounts (120 vs. 240 kg N/ha·a).

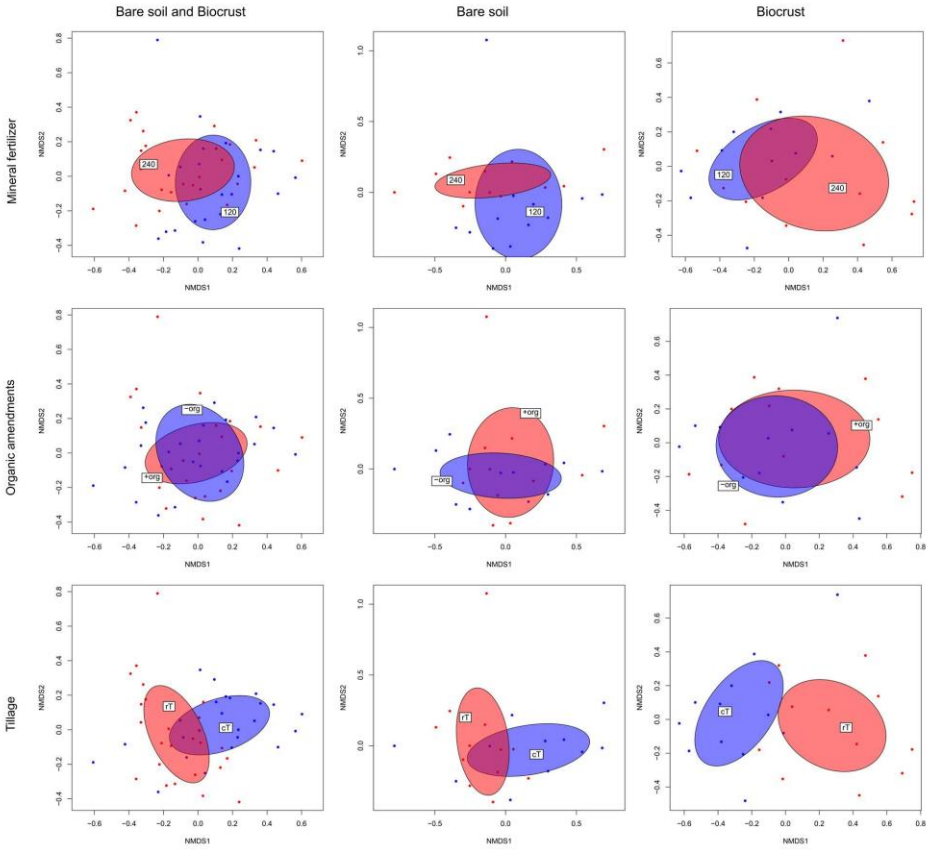

Fig. S4 NMDS plots for prokaryotic community composition at the species level in bare soils and biocrusts colored by treatment (N = 24). Treatments are given for tillage (conventional (cT) vs. reduced tillage (rT)), organic amendments (without (-org) vs. with crop residues (+org)), and mineral fertilization amounts (120 vs. 240 kg N/ha·a).

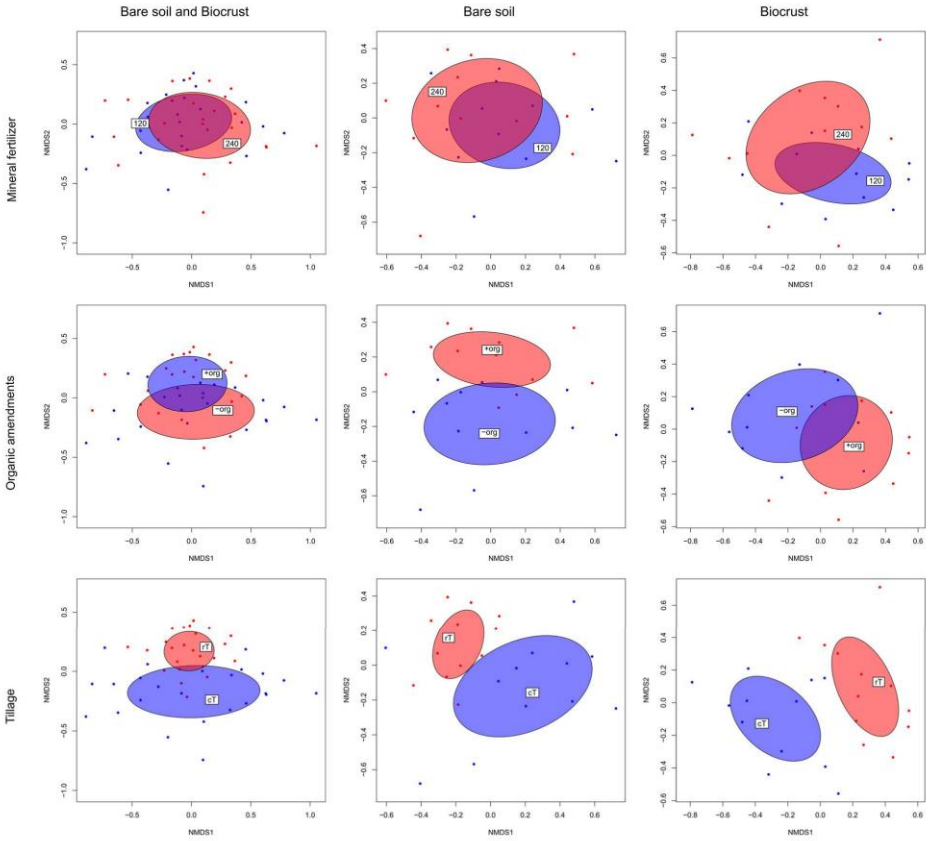

Fig. S5 NMDS plots for fungal community composition at the species level in bare soils and biocrusts colored by treatment (N = 24). Treatments are given for tillage (conventional (cT) vs. reduced tillage (rT)), organic amendments (without (-org) vs. with crop residues (+org)), and mineral fertilization amounts (120 vs. 240 kg N/ha·a).

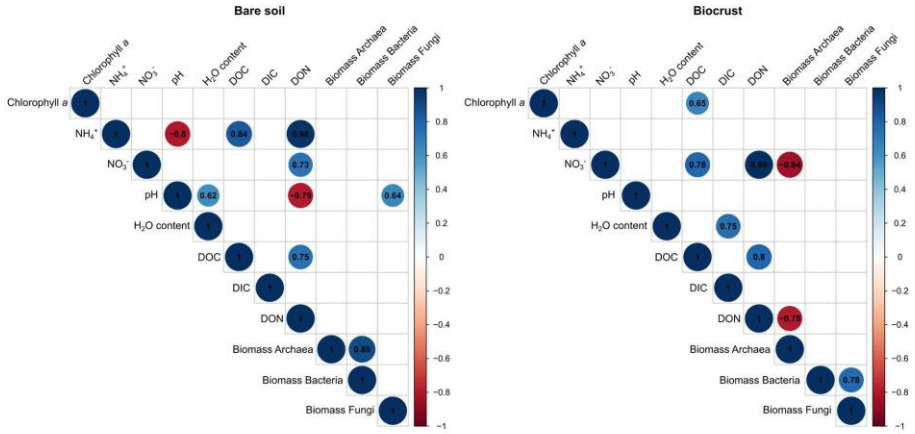

Fig. S6 Significant ($p \leq 0.05$) Pearson correlations ($r > 0.4$ and $r < -0.4$) for bare soils and biocrusts (each N = 24) of soil properties and microbial biomass, where positive correlations ($r > 0$) are shown in blue and negative ones ($r < 0$) are shown in red. R values are given in the circles.

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