environmental microbiology

Environmental Microbiology (2018) **20**(12), 4444–4460 doi:10.1111/1462-2920.14366

Microbial community assembly differs across minerals in a rhizosphere microcosm

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Summary

Mineral-associated microbes drive many critical soil processes, including mineral weathering, soil aggregation and cycling of mineral-sorbed organic matter. To investigate the interactions between soil minerals and microbes in the rhizosphere, we incubated three types of minerals (ferrihydrite, kaolinite and quartz) and a native soil mineral fraction near roots of a common Californian annual grass, *Avena barbata*, growing in its resident soil. We followed microbial colonization of these minerals for up to 2.5 months – the plant's lifespan. Bacteria and fungi that colonized mineral surfaces during this experiment differed across mineral types and differed from those in the background soil, implying that microbial colonization was the result of processes in addition to passive movement with water to mineral surfaces. Null model analysis revealed that dispersal limitation was a dominant factor structuring mineral-associated microbial communities for all mineral types. Once bacteria arrived at a mineral surface, capacity for rapid growth appeared important, as ribosomal copy number was significantly correlated with relative enrichment on minerals. *Glomeromycota* (a phylum associated with arbuscular mycorrhizal fungi) appeared to preferentially associate with ferrihydrite surfaces. The mechanisms enabling the colonization of soil minerals may be foundational in shaping the overall soil microbiome composition and development of persistent organic matter in soils.

Received 22 January, 2018; revised 20 July, 2018; accepted 20 July, 2018. *For correspondence. E-mail twhitman@wisc.edu.

Introduction

Mineral-associated microbes drive many critical soil processes, including mineral weathering (Banfield *et al*., 1999; Uroz *et al*., 2009), metal speciation, toxicity and mobility (Gadd, 2010), aggregate formation (Six and Paustian, 2014) and the cycling of mineral-sorbed organic matter (Schmidt *et al*., 2011; Saidy *et al*., 2014; Kallenbach *et al*., 2016). However, given that 1 g of soil may contain a billion microbial cells, the patchiness of soil microbial communities can be surprising (Raynaud and Nunan, 2014). In most soils, mineral surfaces are not fully colonized by microbes (Ranjard and Richaume, 2001; Nunan *et al*., 2003; Vos *et al*., 2013) and are not 'saturated' with organic matter (Lehmann *et al*., 2007; Kögel-Knabner *et al*., 2008; Miltner *et al*., 2011). Mineral surfaces are constantly being modified or regenerated in surface soils through the dynamic processes of mineral weathering and formation. While the canonical role of lichens in rock colonization and subsequent soil formation is well described (Cooper and Rudolph, 1953; Chen *et al*., 2000; Hodkinson *et al*., 2002; Raab *et al*., 2012), we know little about the first inhabitants of minerals as they form within the soil (Hutchens, 2009).

In addition to the formation of new microhabitats through mineral weathering, frequent disturbances, ranging from large-scale climatic changes (Pold and DeAngelis, 2013) to the millimetre-scale incursion of roots (Belnap *et al*., 2003), regularly 'reset' microscale

communities (DeAngelis *et al*., 2008a, 2008b). These disruptions ensure that meaningful 'stable states' or 'climax communities' are rare, and microbial colonization processes are likely important determinants of soil microbial community composition. Studying microbial colonization of 'fresh' soil minerals (i.e., minerals free of soil organic matter and cells) can provide insight into microbial community assembly in the 'mineralosphere' (Uroz *et al*., 2009) and in soils as a whole. Furthermore, current understanding of the mechanisms behind organic matter persistence in soils includes an important role for mineral-attached microbes (Cotrufo *et al*., 2013; Kallenbach *et al*., 2016). For example, Kallenbach and colleagues (2016) found that microbial soil organic matter accumulation on minerals was greatest in soils with microbial communities characterized by high carbon use efficiency and more fungi. If microbe–mineral interactions are key to soil organic matter stabilization, the dynamics that control and determine which microbes colonize mineral surfaces are also critical.

Surface attachment confers important advantages for soil microorganisms, including protection from predation, access to nutrients or energy sources and provision of a substrate for biofilm formation or other density-dependent phenomena (Hutchens, 2009; Uroz *et al*., 2015). However, soil minerals can provide much more than simply an attachment surface. Different minerals offer specific chemical or physical environments – e.g., varying in surface area, redox status and chemical composition (Banfield and Hamers, 1997) – which may regulate the degree of microbial colonization and even community composition. For example, Hutchens and colleagues (2010) found significantly different bacterial and fungal communities colonized different granitic minerals within the same exposed rocky outcrop. However, relatively few mineral colonization studies have been done in a soil context (Uroz *et al*., 2012). In a volcanic soil using PLFAs and DGGE, Wilson and colleagues (2008) found that while some minerals were more intensively colonized, magnetically separated Fe/Mg minerals *versus* volcanic glass or K-feldspar minerals had similar microbial (bacterial, archaeal and fungal) community compositions. Similarly, Berner and colleagues (2012) determined that the community of ectomycorrhizal fungi that colonized apatite-amended sand did not differ from unamended sand, although the total biomass was greater in the apatite treatment, using a buried mesh bag and clone library approach. In contrast, Carson and colleagues (2009) found different minerals (mica, basalt and rock phosphate) selected for different bacterial communities in a planted (ryegrass or clover) and unplanted Haplic Podzol using an ARISA approach. Using a clone library approach, Uroz and colleagues (2012) also found that different bacterial communities colonized minerals (pure apatite, pure plagioclase and a mix of phlogopite-quartz) after 4 years of burial in acidic forest soils (Typic Dystrochrepts). Using high-throughput sequencing to analyse samples from the same trial, they confirmed that *Burkholderia* was a key genus responsible for apatite weathering in soils (Lepleux *et al*., 2012). At the same site, Colin and colleagues (2017) found strong differences in bacterial communities on buried minerals of varying weatherability (apatite, obsidian and calcite) compared to the bulk soil after two and a half years. Additionally, they found that bacterial taxa associated with mineral-weathering capabilities (from the *Betaproteobacteria* class and *Burkholderia* and *Collimonas* genera) were more abundant on the less easily-weathered mineral types (obsidian and apatite). Ahmed and colleagues (2016) have also reported significant differences in microbial (bacterial, archaeal and fungal) communities, using solid mineral sheets (apatite, biotite and oligoclase) buried in a Haplic Podzol over 2 years using high-throughput sequencing. These previous studies clearly show that different minerals often host distinct soil microbial communities, although the effects of mineralogy on microbial colonization of fresh minerals and, in particular, the mechanisms that control their community assembly processes are still being identified (Uroz *et al*., 2015). In our study, we explored these phenomena within a rhizosphere context, where altered chemical and resource characteristics from proximity to plant roots create a unique environment (Kuzyakov and Blagodatskaya, 2015).

The factors that shape community assembly (Drake, 1990; Keddy, 1992; Tilman, 2004; Maherali and Klironomos, 2007; Zhou and Ning, 2017) and community succession within new habitats (Gleason, 1939; Young *et al*., 2001; Hodkinson *et al*., 2002) have long been studied for macrobiota. Similar principles may be applied to understand the assembly of microbial communities. For example, in a fluid ecosystem, Zhou and colleagues (2014) evaluated the concepts of deterministic *versus* stochastic processes, showing that both processes played important roles, but their relative importance varied over time. In our study of microbe-scale colonization of fresh mineral surfaces within the rhizosphere, we considered a number of central questions. Are community members limited by dispersal, or are new mineral habitats readily accessible? Once microbes arrive at a new microhabitat, does selection favour certain organisms from the source community or is a novel community drawn indiscriminately from the surrounding rhizosphere soil? To address these questions, we investigated which microbes colonize 'fresh' mineral surfaces in the soil, what community assembly processes determine initial community composition in the mineralosphere, and the characteristics that make microbes strong colonizers of mineral surfaces in rhizosphere soil. Our null hypothesis was that mineral community composition would largely resemble those of the background soil – i.e., they would be structured largely by stochastic processes. We incubated fresh minerals commonly found in our study soil (quartz, kaolinite and ferrihydrite) as well as

density-fractionated native soil minerals ('heavy fraction') in soil microcosms with the annual grass *Avena barbata* (Pott ex Link) for up to 2.5 months (Fig. 1), planted at a density where all the soil was effectively 'rhizosphere'. We then assessed how different minerals selected for distinct fungal, bacterial and archaeal communities in rhizosphere soil using high-throughput sequencing. We used null models to test whether similarities or differences in the microbial composition of the fresh minerals *versus* that of the surrounding soil were consistent with homogenizing selection, homogenizing dispersal, variable selection or dispersal limitation being the dominant processes structuring communities.

Results

Mineral colonization

We extracted significantly more DNA from whole (rhizosphere) soil than from all mineral types and the heavy fraction (Fig. 2). Of the minerals, we extracted significantly more DNA from ferrihydrite and the heavy fraction and the least from kaolinite. (Any kaolinite samples that had DNA extraction and amplification levels below blank controls were excluded from our analyses.) These trends were generally mirrored in 16S rRNA gene copies (bacterial and archaeal) and ITS copies (fungal), as determined by qPCR (Supporting Information Figs S1 and S2), although due to issues with the method, we caution against interpreting these trends too strongly (see Supporting Information Note S1). These trends remained similar when considered on a mass basis (Supporting Information Figs S1 and S2). Still, we note that there is no optimal metric by which to compare the likely microbial colonization of the minerals. On one hand, a mass basis obscures orders of magnitude-large differences in surface area across the minerals. On the other hand, a surface-area basis is limited by the methods of measuring surface area – specifically, BET surface area likely detects surface area at much finer scales than would be microbially accessible/relevant. However, we expected this information would be of interest to some readers, and note that the broad trends (lowest DNA or gene copies on kaolinite) persist under both metrics.

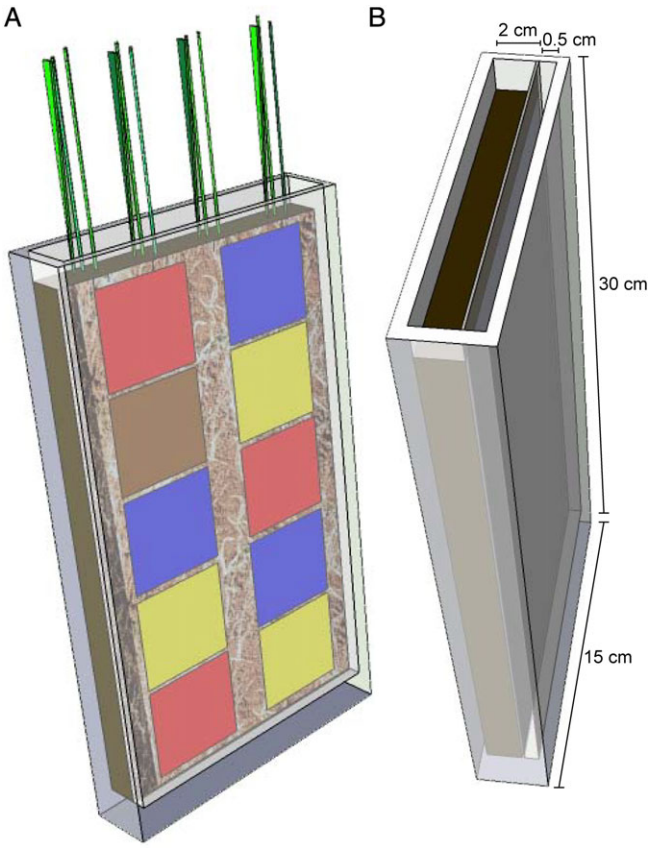

Fig. 1. Microcosm design. Ferrihydrite, kaolinite and quartz minerals with pure, C-free surfaces and density-separated soil minerals (represented as coloured squares) were incubated adjacent to rhizosphere soil in microcosms with growing *Avena barbata* (A). Plants were grown for 1 month before mineral bags were added, with randomized locations in each microcosm. Space was reserved for the minerals using a removable side panel (B). After removing the side panel and adding minerals, they were covered with soil (not pictured). [Color figure can be viewed at wieyonlinelibrary.com]

Community composition

Across samples, the most abundant bacterial phyla were *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, *Firmicutes* and *Acidobacteria* (Fig. 3). The mineral microbial communities had significantly lower relative abundances of *Acidobacteria*, *Planctomycetes*, *Chloroflexi* and *Gemmatimonadetes* than the soil communities. On average, archaea made up only 0.02% of all 16S reads across all samples. The most abundant archaeal phyla were *Crenarchaeota* and *Euryarchaeota.*

Fungal communities were dominated by *Ascomycetes* and *Basidiomycetes*, although large fractions (up to 40% in kaolinite minerals) were not identifiable using the UNITE database (Kõljalg *et al*., 2013), even at the phylum level. At a finer taxonomic level, the most abundant orders (for those taxa identifiable to order) were *Sordariales*, *Eurotiales* and *Hypocreales.* The orders *Sordariales* and *Eurotiales* had significantly lower relative abundances in the minerals as compared to the soils, and orders *Sebacinales* and *Glomerales* had significantly higher relative abundances in the ferrihydrite minerals than the soils (Fig. 4 and Supporting Information Fig. S3).

Community comparisons

Community composition Bray–Curtis dissimilarities differed significantly by soil and mineral type (Fig. 5) for both fungi and bacteria/archaea (permutational multivariate ANOVA,

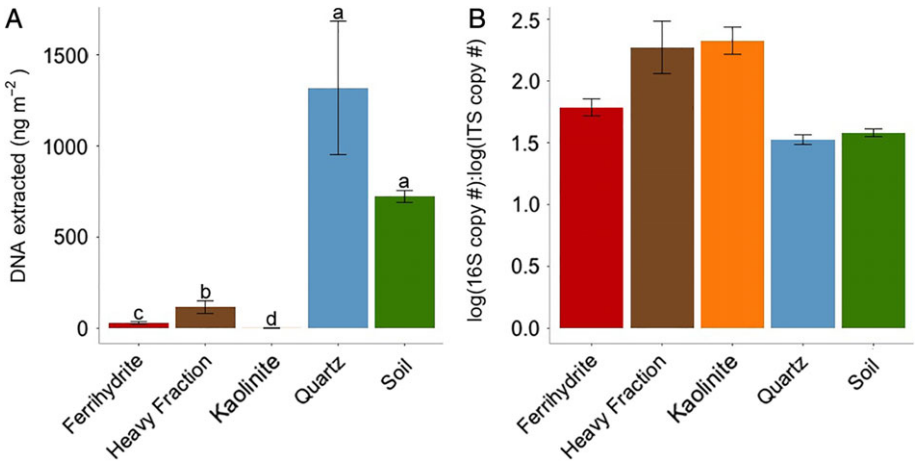

Fig. 2. (A) Total DNA extracted from soils and minerals, normalized by surface area (bulk soil surface area was not measured, so heavy fraction mineral surface area was used to generate this figure), and (B) log(16S rRNA gene):log(ITS) copy number ratios. Mean values after 2–2.5 months after the start of the experiment, with error bars representing ±SE (n = 3 for heavy fraction, n = 8–23 for all other minerals). Lowercase letters indicate significant differences (p < 0.05, ANOVA, Tukey's HSD). [Color figure can be viewed at wieyonlinelibrary.com]

p < 0.001). There was not a significant effect of microcosm (permutational multivariate ANOVA, p < 0.38) for bacteria/archaea, but there was for fungi (permutational multivariate ANOVA, p < 0.02) so we controlled for microcosm when it was significant. While there were significant changes in community composition over time for fungi (p < 0.005), these were not large and were not significant for bacteria/archaea (p < 0.08). For the remaining analyses, we present results from the 2 and 2.5 month time points combined, as they were not significantly different in community composition (p < 0.12 for both fungi and bacteria/archaea), had similar levels of diversity (Supporting Information Fig. S4) and had more DNA extracted than the 1 month time point (Supporting Information Figs S5 and S6).

The relative abundance of taxa within mineral-associated microbial communities differed from that of the soil community. In the bacteria/archaea, 39% of all OTUs were significantly enriched in at least one mineral as compared to the soil, while 27% were significantly depleted in at least one mineral. *Acidobacteria* tended to be depleted in the ferrihydrite and the quartz minerals, while *Firmicutes* and *Bacteroidetes* tended to be enriched (Fig. 3 and Supporting Information Fig. S8). *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia* showed both positive and negative responses. In the fungi, 9% of all OTUs were significantly enriched in at least one mineral as compared to the soil while 14% were significantly depleted in at least one mineral. *Glomeromycota*, the arbuscular mycorrhizal fungi, were consistently enriched in relative abundance in the ferrihydrite minerals, but not the quartz or kaolinite (Fig. 4 and Supporting Information Fig. S9), while there was a broader range of responses for the *Ascomycota* and *Basidiomycota.*

In order to examine these responses at a finer phylogenetic resolution, we plotted the OTUs that were significantly (FDR < 0.1) enriched in minerals as compared to the soil (4× or greater – $\log_2$-fold change = 2), grouped by family (Supporting Information Fig. S10). Of these taxa, *Burkholderiaceae*, *Chitinophagaceae*, *Comamonadaceae*, *Phyllobacteriaceae*, *Rhizobiaceae*, *Rhodospirillaceae* and *Streptomycetaceae* were enriched in both the ferrihydrite and quartz minerals, while *Bacillaceae* were only enriched in the quartz minerals. The 10 most enriched OTUs in each phylum for each mineral are provided in Supporting Information Data S1. Within this table, OTUs common to all three minerals were identified as *Rhodococcus fascians* and *Staphylococcus* sp. The most abundant mineral-enriched taxa in ferrihydrite included a *Chitinophaga* sp., *Rhizobium* sp. and *Caulobacter henricii.* In quartz, the most abundant mineral-enriched taxa were *Chitinophaga* sp., *Janthinobacterium* sp. and *Nevskia* sp. In kaolinite, the top two were identified only to family (*Rhizobiales*) and the third was a *Dechloromonas* sp. We also considered the OTUs from the phylum *Acidobacteria* by subgroup and found that subgroups 1, 10, 17, 6 and 7 tended to be depleted in ferrihydrite and quartz relative to the soil, while for kaolinite, subgroups 10, 6 and 7 were also generally depleted and subgroups 2 and 3 were enriched relative to the soil (Supporting Information Fig. S11).

We were not able to taxonomically resolve the fungi as well as the bacteria and archaea. The ITS2 region diverges at too fine of a genetic scale to match ITS2 sequences to phylogenetic levels coarser than species level or genus level. Thus, for a large fraction (> 50% in some samples) of responding taxa we were not able to assign taxonomy. Of the identified taxa, OTUs that matched *Serendipita* and *Pochonia* genera in the UNITE database (Kõljalg *et al*., 2013) both stood out as strong responders in both ferrihydrite and quartz minerals (Supporting Information Fig. S12). OTUs that best matched *Agaricales* were also enriched in quartz, while OTUs that best matched *Sebacinales* were also enriched in ferrihydrite. *Trichosporon* was identified as

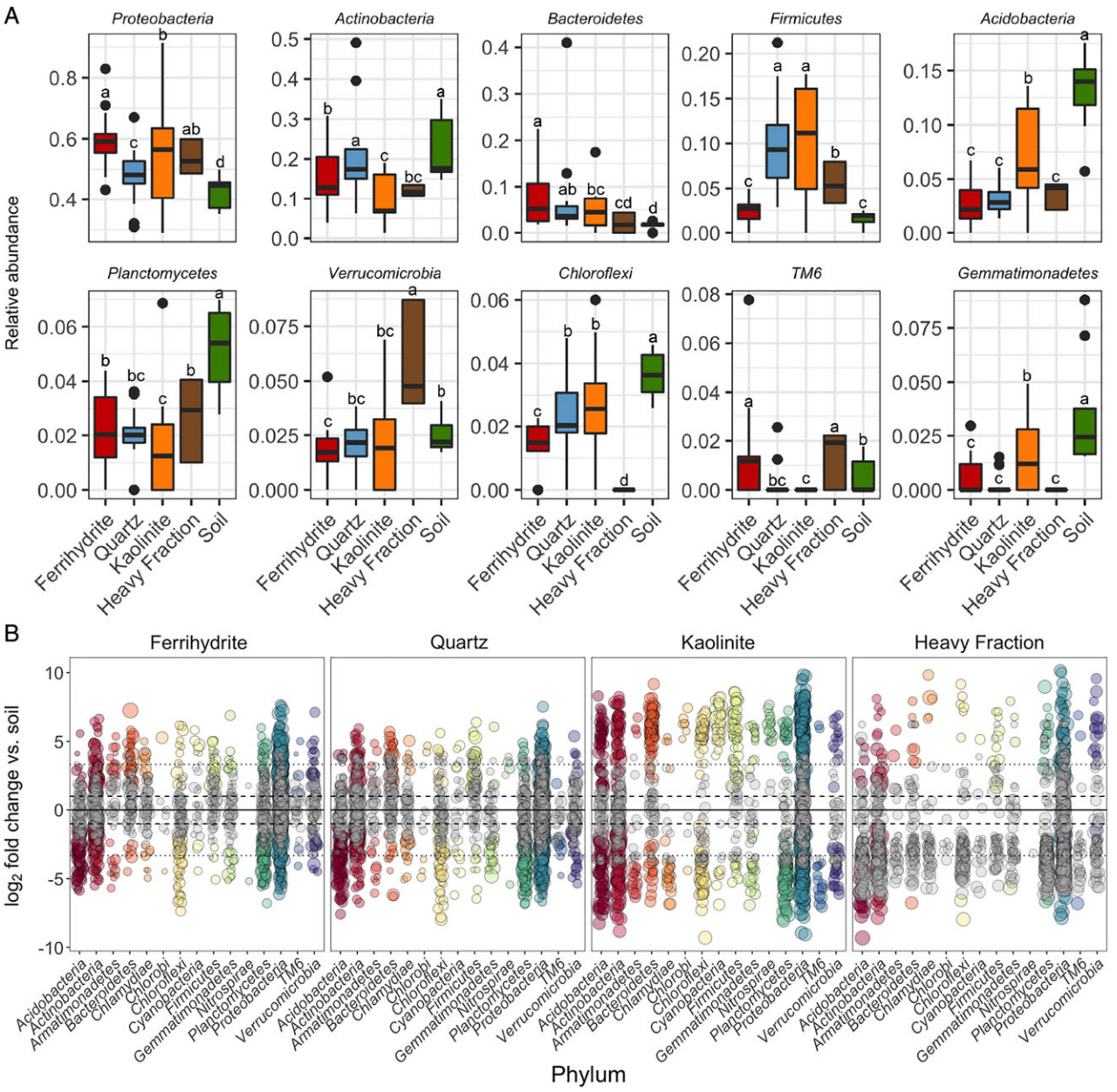

Fig. 3. (A) Relative abundance of top 10 bacterial phyla in different mineral types. For heavy fraction, $n = 3$; for all others $n = 11–14$. Letters indicate significant differences within a phylum ($p < 0.05$, ANOVA, Tukey's HSD). (B) Log_2-fold change in relative abundance of bacterial OTUs in mineral samples *versus* soil, after 2–2.5 months of exposure to roots and soil. Dashed line represents a 2× change, while dotted line indicates a 10× change. Each point represents the mean response for a single OTU, and the size of the point represents the log(relative abundance). Only OTUs with an FDR < 0.1 are shown in colour, with remaining OTUs shown in grey. Heavy fraction minerals were only sampled at 2 months. [Color figure can be viewed at wieyonlinelibrary.com]

being enriched in the kaolinite minerals. The 10 most enriched OTUs in each phylum for each mineral are provided in Supporting Information Data S2. Within this table, there were no enriched OTUs common to all three minerals. The three most abundant mineral-enriched taxa in ferrihydrite included a *Pochonia* sp., an unidentified Ascomycete and an unidentified OTU. In quartz, the most abundant mineral-enriched taxa were identified only to family (*Agaricales*) or class (two *Dothideomycetes*). In kaolinite, the three most abundant taxa were not identifiable through the UNITE database.

Predicted 16S rRNA gene copy number was significantly correlated with log_2-fold change in relative abundance in minerals *versus* soil, controlling for phylum and mineral type (ANOVA, $p < 0.0001$, $R^2_{adj} = 0.28$). There were not significant interactions between mineral type and predicted 16S rRNA gene copy number.

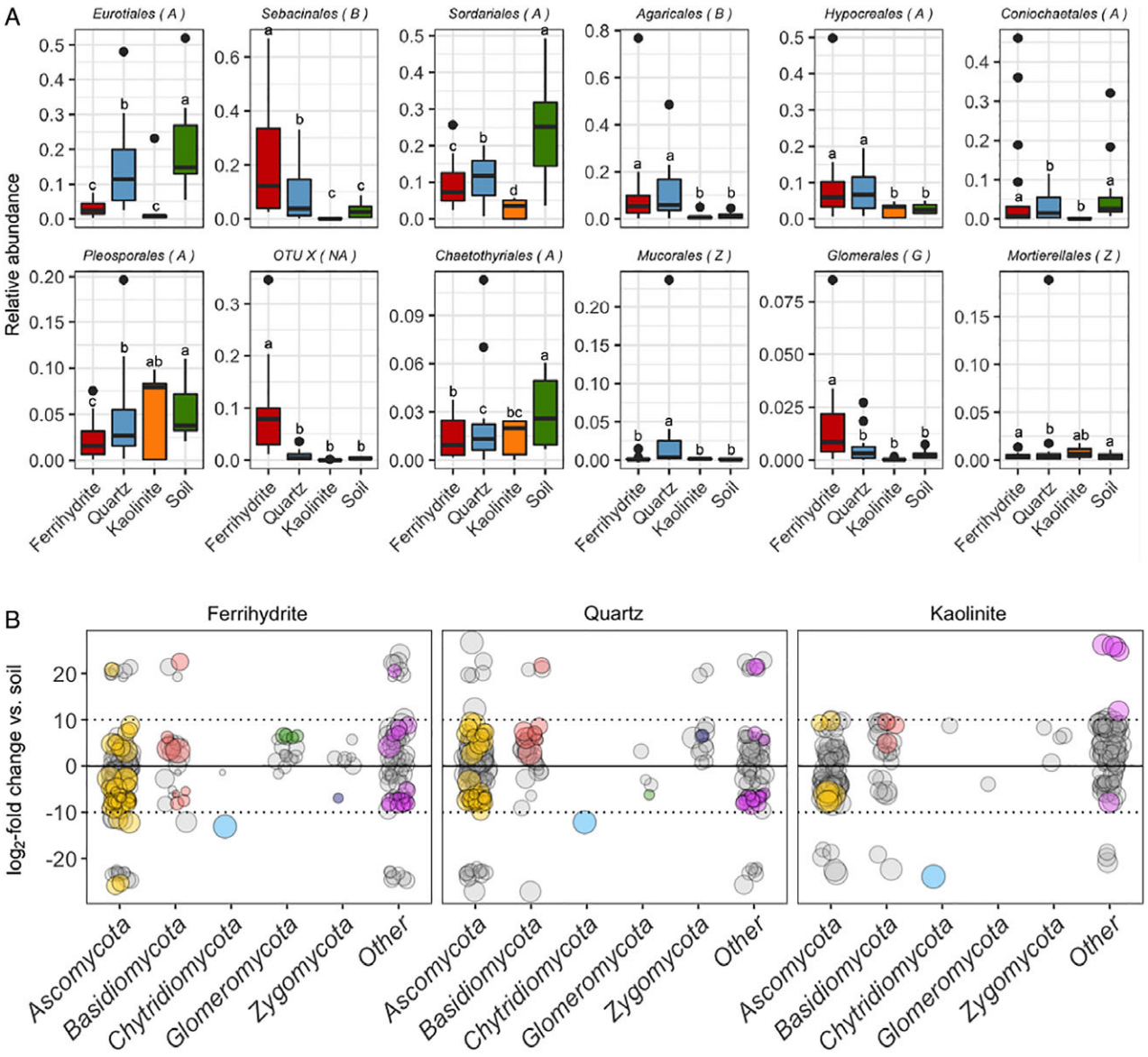

Fig. 4. (A) Relative abundance of top 10 fungal orders in different mineral types. Phylum is indicated in brackets: A = *Ascomycota*, B = *Basidiomycota*, G = *Glomeromycota* and Z = *Zygomycota.* For kaolinite, $n = 4$; for all others $n = 10–14$. Letters indicate significant differences within an order ($p < 0.05$, ANOVA, Tukey's HSD). We were not able to amplify and sequence ITS2 amplicons from DNA extracted from the heavy fraction at levels greater than the control/blank extractions, so those data are not shown. (B) Log_2-fold change in relative abundance of fungal OTUs in mineral samples *versus* soil, after 2–2.5 months of exposure to roots and soil. Each point represents the mean response for a single OTU, and the size of the point represents the log(relative abundance). Only OTUs with an FDR < 0.1 are shown in colour, with remaining OTUs shown in grey. We were not able to amplify and sequence ITS2 amplicons from DNA extracted from the heavy fraction at levels greater than the control/blank extractions, so those data are not shown. [Color figure can be viewed at wieyonlinelibrary.com]

Community assembly

To determine the dominant processes that may be structuring communities in the minerals and rhizosphere soils, we used the approach described by Stegen and colleagues (2013) to classify ecological processes into the following categories: homogeneous selection, variable selection, homogenizing dispersal, dispersal limitation and undominated (see 'Experimental procedures' and Stegen *et al*., 2013, 2015). The dominant process predicted to be governing community assembly across the soil bacterial and archaeal communities within a given time point was homogenizing selection (i.e., abiotic or biotic pressures select for closely related taxa from one soil sample to the next; Fig. 6). Homogenizing selection was also predicted to have played a role in the assembly of quartz and ferrihydrite communities as compared to soil communities. Interestingly, dispersal limitation was predicted to have played a dominant role in

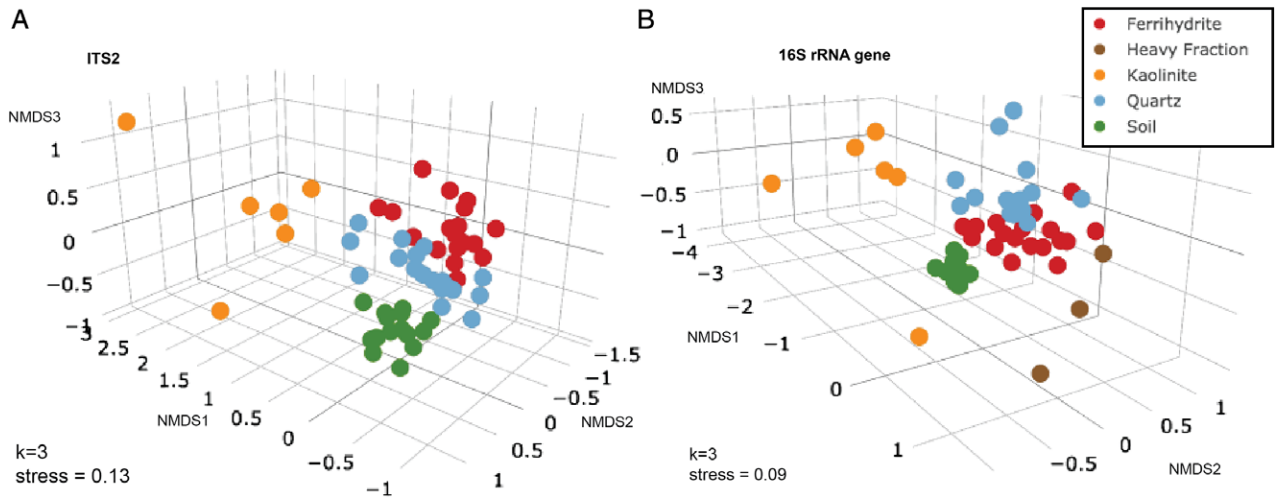

Fig. 5. Three-dimensional NMDS plots of Bray distances for (A) fungal ITS2 (k = 3, stress = 0.13) and (B) bacterial/archaeal 16S rRNA gene (k = 3, stress = 0.09) communities, along with bulk soil samples. We were not able to amplify and sequence ITS2 amplicons from DNA extracted from the heavy fraction at levels greater than the control/blank extractions, so those data are not shown. [Color figure can be viewed at wieyonlinelibrary.com]

controlling community assembly on all mineral surfaces (Fig. 6). However, in contrast to other mineral surfaces, variable selection was predicted to have played a role in governing microbial community structure on kaolinite minerals (Fig. 6).

Discussion

Possible mechanisms of microbial colonization of fresh minerals

Our null hypothesis was that there would be no meaningful dispersal limitations or selective pressures associated with mineral colonization. The null hypothesis would be consistent with microbes being swept passively onto the minerals with the movements of soil water, and we would have expected that the resulting communities should largely resemble those of the source soil. However, based on this quantitative approach (Stegen *et al*., 2013), we found that dispersal limitation was an important factor shaping the differences of mineral communities from soil communities, for all mineral types (Fig. 6) – that is, in one growing season of an annual grass (2.5 months), a large portion of soil microbes will not be expected to reach the minerals by neutral dispersal. Thus, we hypothesize that while this may simply indicate that spatial proximity to minerals may be a key factor in successful colonization, some of the first successful colonizers might be capable of active movement to the minerals. This would require the expression of flagella or other motility factors, such as swarming (Dechesne *et al*., 2010) and sufficient soil water to support bacterial movement. In addition, water movement by diffusion or advection into and out of the mineral bags could have differed between bags and

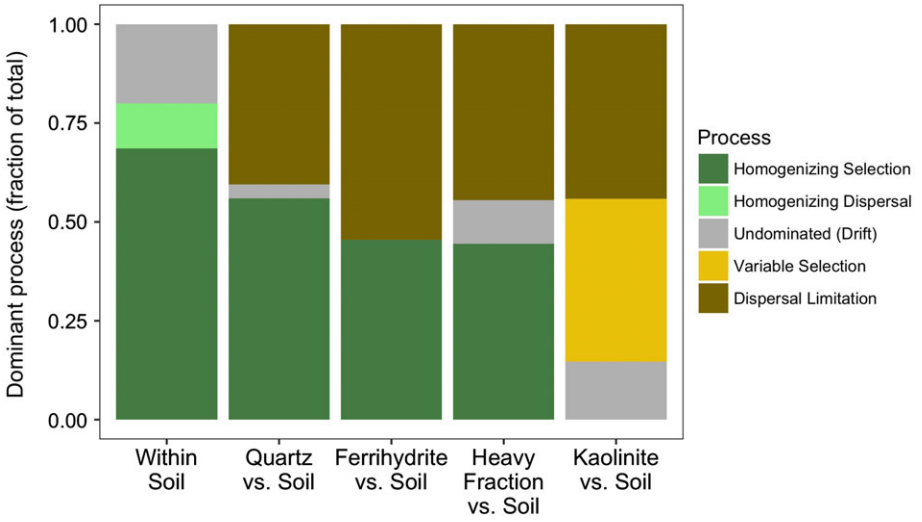

Fig. 6. Relative influence of different bacterial/archaeal community assembly processes on spatial turnover among soil communities and between soil communities and those on different mineral surfaces. The governing processes were determined using RC_{Bray} and βNTI (Stegen *et al*., 2013). Different colours represent different fractions of community turnovers governed by each process. [Color figure can be viewed at wieyonlinelibrary.com]

between bags and the soil. Thus, differences in patterns and processes of water movement may also be responsible for some of the observed differences. In addition to moving freely through water, it has been shown that some bacteria or archaea are also be able to move on 'fungal highways' in soil, where bacteria move or are carried along fungal hyphae to environments they would otherwise be unable to colonize (e.g., Simon *et al*., 2017). In an intriguing *in situ* experiment with three different soil types, Simon and colleagues (2017) found that *Mortierella* were the predominant fungal taxa to colonize media across air gaps. Additionally, they identified numerous bacteria from various phyla (notably *Firmicutes*, *Planctomycetes* and some *Proteobacteria*) that were able to travel on these fungal highways. In agreement with their results, we found that a *Mortierella* OTU was among the most enriched in the quartz mineral bags (although it was not significantly enriched in the other minerals). Although the most abundant bacterial genera from their study were not necessarily the same as those in our study, their results suggest one mechanism – fungal highways – that could help explain the strong enrichment of *Firmicutes* in all minerals. Finally, it should be noted that there are several underlying assumptions of our quantitative approach to community assembly mechanisms (Stegen *et al*., 2015) – violating any of these assumptions could lead to wrong or severe biases of the estimated ecological processes (Zhou and Ning, 2017). While there could be considerably large differences between the statistically measured values and true values of ecological processes, this quantitative framework should be useful for comparative purposes. Such comparisons should be meaningful on a relative basis if all communities are treated in the same way (Zhou and Ning, 2017).

Once microbes have arrived at fresh mineral surfaces, fast-growing microbes may be favoured, more easily winning the competition for new surface area (Converse *et al*., 2015). Although the mineral surfaces in this study were likely far from fully occupied by microbes, our data do support the suggestion that some of the first colonizers may be fast growers. There was a significant positive correlation between enrichment on minerals and predicted 16S rRNA gene copy number. 16S rRNA gene copy number has been linked to fast-growth strategies (Goldfarb *et al*., 2011) and early succession (Nemergut *et al*., 2016). This could provide a second explanation, in addition to the fungal highways hypothesis, for the enrichment of a substantial portion of *Firmicutes* OTUs (Fig. 3 and Supporting Information Fig. S8).

While the chemical environment in the minerals in this study might be expected to reflect that of the soil (which was essentially all rhizosphere) in its relative composition, the total amount of C and other nutrients available on the minerals were likely much lower than in the soil. One source of nutrients and energy could be the first colonizers of the minerals themselves: OTUs identified as possible predatory bacteria such as *Cytophaga* and *Bdellovibrio*, or the possible fungal predator/endosymbiont *Chitinophaga* (Shaffer *et al*., 2017) were consistently and sometimes much higher in relative abundance in minerals (Supporting Information Note S2 and Supporting Information Fig. S13).

Another way to survive in sparse environments could be to access resources from elsewhere via filamentous growth. Significant fungal colonizers of minerals included mycorrhizal symbionts (Fig. 4 and Supporting Information Fig. S14). Unlike saprotrophic fungi, mycorrhizae have access to a direct plant-derived C source and so can possibly better 'afford' to explore the low-C mineral environments. Supporting this idea, we found that AMF were enriched in ferrihydrite minerals (Fig. 4 and Supporting Information Fig. S14), and *Sebacinales* and *Serendipita vermifera* (possible mycorrhizal fungi) were significantly enriched in ferrihydrite and quartz (Supporting Information Fig. S12). Unlike AMF, we found that fungi classified as predicted saprotrophs (Nguyen *et al*., 2016) tended to be significantly depleted in ferrihydrite (Supporting Information Fig. S14), likely because there was little C on which they could subsist. However, while we might have predicted that, due to their ability to draw on resources elsewhere through their hyphae, fungi would generally be better colonizers of the sparse mineral environments than bacteria/archaea, this was not supported by the qPCR data. Compared to soils, no significant differences were observed in the 16S rRNA gene *versus* ITS copy number ratios in quartz or ferrihydrite and the heavy fraction and kaolinite minerals actually had significantly higher 16S rRNA gene *versus* ITS copy number ratios than were found in soils (Fig. 2). Even so, copy number can vary greatly within bacteria, and become even more tenuous in multinucleate fungi, so this data should only be interpreted with caution. In addition, if filamentous growth alone offers a colonization advantage, we might predict that *Actinobacteria* would tend to be enriched in minerals. However, this was not observed. Thus, we did not find evidence that filamentous growth alone serves as a reliable predictor of greater colonization success – a robust C source (such as that secured through symbiosis) may also be required. In a soil mineral colonization study, Ahmed and colleagues (2016) suggested that the increased abundance of *Chytridiomycota* on oligoclase surfaces may have been due to an increased presence of its protozoan and metazoan hosts on that mineral compared to other, less-colonized minerals. Somewhat similarly, in our experiment, the OTU identified as *Chitinophaga* sp. was one of the most mineral-enriched OTUs ($\log_2$-fold changes of 7.3 and 4.0 in ferrihydrite and quartz, respectively, and representing > 1% of the total

community in ferrihydrite) and could be a possible consumer or endosymbiont of fungi (Shaffer *et al*., 2017). Thus, the first colonizers may serve as a nutrient resource for subsequent colonizers of the minerals.

In addition to dispersal limitation, homogeneous selection was an important factor for all minerals except for kaolinite, which was structured by variable selection in relation to the soil communities (Fig. 6). While the explanation for homogeneous selection is likely relatively straightforward – certain features of quartz and ferrihydrite resemble those of the soil, and result in similar environments with similar selective pressures – there may be a few possible explanations for the variable selection in kaolinite. These explanations could include various ways in which the kaolinite environment was more different from that of the background soil than were the quartz and ferrihydrite environments. For example, much higher surface area, smaller particle size and lower pH (Table 1) may have created a kaolinite environment that was substantially different from that of the background soil. A comparatively sparse distribution of resources on kaolinite minerals, given their high surface area, could have resulted in stronger selective pressure for arriving microbes, also contributing to the greater importance of variable selection and competition in these communities (Fig. 6). Additionally, the difference in the environments inside *versus* the outside of the mineral bags may have been greater for kaolinite than for the other minerals, due to its very small particle size, further differentiating the kaolinite environment. This highlights the importance of considering these minerals and our findings not only in the context of their mineralogy, but also the effect of their particle size and surface areas, which affects factors such as weathering rates (Turpault *et al*., 2008; White, 2002) and microbially habitable surface (Minyard *et al*., 2012).

Our study examined the initial stages of microbial colonization on minerals over a single growing season. Future work following these trends over a longer period of time could address whether dispersal limitation plays a meaningful long-term role in structuring soil mineral communities – do the bacteria that arrive first continue to prevail in the community? This hypothesis may be supported by the observation that the bacterial communities in minerals were more variable than those in the bulk soil (Fig. 5) – suggesting that there is variability in which specific microbes happen to first colonize the fresh minerals. However, only future studies spanning multiple plant growing seasons could determine how long these assemblages might persist and whether the arrival and establishment of the first sets of microbes could result in exclusion of future potential colonists, or whether the minerals would quickly come to resemble the bulk soil community. An additional confounding factor to this study is that the soil community represented by ribosomal DNA is an integrated profile of the historical soil microbial community, not just active, or even living, microbes (Blazewicz *et al*., 2013; Carini *et al*., 2016). After years in the soil, cycling through disturbances and environmental changes, the apparent (historical) diversity of microbes on the minerals would also be expected to increase, simply as the microbial record of environmental change accumulates. However, bulk soil has more diverse mineralogy than the homogeneous minerals, and thus, more diverse microenvironments, so some of these differences may persist.

Table 1. Mineral properties.

Property	Quartz	Pure kaolinite[a]	Ferrihydrite	Heavy fraction[b]
Chemical formula	SiO_4	$Al_2Si_2O_5(OH)_4$	$Fe(OH)_3$	NA
Source of mineral	Purchased from Sigma-Aldrich (274739)	Kaolinite purchased from the Clay Minerals Society (K-Ga2)	Synthesized in lab following Hansel and colleagues (2003)	Extracted from soil using modified protocol from Sollins and colleagues (2006)
BET surface area[c] ($m^2\ g^{-1}$)	0.01–0.05[d]	20.48	4.8	2.68
Particle size range	297–210 μm	Mostly < 2 μm	Coated quartz	Not determined
pH (1:1 m:v in 0.01 M $CaCl_2$)	4.12	3.03 (kaolinitequartz mix)	6.23	Not determined
Initial C (%)	Negligible	Negligible	Negligible	1.6
Hydroxylamine-extractable Al (mean ± SE, $\mu g\ g^{-1}$)	8 ± 2	8.4 ± 0.4 (kaolinitequartz mix)	4563 ± 1535	Not determined
Hydroxylamine-extractable Fe (mean ± SE, $\mu g\ g^{-1}$)	1.5 ± 0.1	77 ± 14 (kaolinitequartz mix)	587 ± 268	Not determined
Predicted relative charge density	Very low	Low	High	Intermediate
Primary or secondary mineral?	Primary	Secondary	Secondary	NA

a. Kaolinite was used in a 50:50 mixture with quartz.
b. Numerous parameters were not determined due to very low quantities.
c. N_2 analysis gas.
d. Quartz surface area was too low to measure using the above techniques with N_2 gas. Estimated from the literature (Xu *et al*., 2009; Mekonen *et al*., 2013) and mesh size.

Mineral specificity in microbial community assembly

Our results from a rhizosphere soil were consistent with previous studies of minerals in other environments (Gleeson *et al*., 2005; 2006; Wilson *et al*., 2008; Carson *et al*., 2009; Hutchens *et al*., 2010; Uroz *et al*., 2012; Ahmed *et al*., 2016), in that different minerals harboured significantly different bacterial/archaeal and fungal communities (Figs 3–5), with evidence for selection based on phylogenetic lineage (Supporting Information Figs S15 and S16) – i.e., communities have a stronger phylogenetic signal than would be expected by chance. Microbial colonization may have been highest in the ferrihydrite minerals, as we were able to extract significantly more DNA from ferrihydrite than the quartz or kaolinite on a surface area as well as a mass basis (Fig. 2 and Supporting Information Figs S1, S2, S5 and S6). The suggestion that ferrihydrite was more readily colonized by microbes is also consistent both with other studies, which showed increased microbial biomass on Fe-containing minerals (Wilson *et al*., 2008). In addition, the mineral morphology could potentially accentuate the differences in surface accessibility – the platy structure of clay would stack very differently than the granular ferrihydrite or quartz. In addition to physical differences, chemical differences such as pH were likely important factors contributing to different community compositions in the minerals (Table 1 and Supporting Information Table S1). pH has consistently been reported as playing a central role in determining soil microbial community composition, with different subgroups within the phylum *Acidobacteria* associated with different pH responses (e.g., Bartram *et al*., 2013; Kielak *et al*., 2016; Lauber *et al*., 2009; Rousk *et al*., 2010). Considering the subgroup-level patterns of enrichment of the *Acidobacteria* phylum (Supporting Information Fig. S12), the depletion of subgroups 10, 6 and 7 across minerals is consistent with low-pH environments, and the enrichment of subgroups 2 and 3 in kaolinite may point particularly strongly to a low pH, which is consistent with kaolinite having the lowest pH (Table 1). The significant differences in the microbial communities that colonize different minerals suggest that mineralogy and mineral particle size or surface are in natural soils may also be important in determining microbial community structure, with potential implications for biogeochemical cycling and persistence of soil organic matter.

Speculations on C stabilization and microbial mineral colonization

Our findings could possibly have implications for C stabilization mechanisms in the soil (although the following discussion should be seen as highly speculative at this point). For example, others have found significant positive correlations between C accumulation rates and carbon use efficiency of microbial communities (Kallenbach *et al*., 2016; Wang *et al*., 2017). If we could identify or predict the carbon use efficiencies of specific taxa, we may be able to predict how the first mineral colonizers will affect C persistence. For example, Wang and colleagues (2017) found that *Acidobacteria* were significantly negatively correlated with both carbon use efficiency and with C accumulation. We found that *Acidobacteria* were often significantly depleted in our minerals, while the opposite trends were observed for *Bacteroidetes* (Fig. 3). This could evoke the following two contrasting hypotheses: (i) the microbes that help stabilize or retain soil C (through their metabolic activities and characteristics such as carbon use efficiency) are also good mineral colonizers and (ii) the first microbes to colonize minerals are not particularly 'adept' at stabilizing C, but simply because they are the first microbes to colonize the fresh mineral surfaces, they contribute directly to relatively stable mineral-associated C through their necromass when they die. This close association with the minerals could also result in preferential preservation of their DNA, explaining their apparent association with high-C soils. These hypotheses could form the basis of interesting future experiments.

To take these speculations even further, if different taxa are more or less likely to produce C stabilization on minerals, then the mechanisms that determine which microbes colonize fresh mineral surfaces are also important. For example, if mineral communities are limited by dispersal, then the carbon use efficiency of the strong dispersing organisms that form the first communities could determine future levels of C stabilization. An excellent candidate for these types of phenomena is *Caulobacter* – *Caulobacter* sp. can form a stalked, attached cell or a flagellated, motile cell (Scott and Ely, 2016). The flagellated form might be especially adept at dispersing to the fresh mineral surfaces, while the stalked form could form a close association once there, possibly contributing to mineral-associated C. In our experiment, one OTU identified as *C. henricii* (100% ID to sequenced *C. henricii* strain CB4; Scott and Ely, 2016) was significantly enriched (32×) in the ferrihydrite minerals and made up ~ 2% of the total community. However, the question remains why it was not as enriched on the other minerals in this study. Again, these are currently speculations and experiments would need to be designed to explicitly test these hypotheses – a first step could be to directly relate CUE of individual taxa to their colonization aptitude and their relative contribution to persistent microbially derived SOC.

Conceptual model

While microbial communities associated with the fresh minerals broadly resembled the source soil communities

(Figs 2–5), significant phylogenetic differences between mineral and soil communities reveal that community assembly on fresh soil minerals is governed by multiple processes. While we expect passive transport of microbes to fresh mineral surfaces by soil water movements occurs, some microbes are likely actively moving or transported to minerals, possibly on hyphal 'highways' (Fig. 6). Once they encounter the minerals, certain microbes become significantly enriched on the new mineral surfaces (Figs 3 and 4), due to a wide variety of possible biological, geochemical and physical drivers (Fig. 7). These mechanisms controlling the colonization of mineral surfaces help govern the overall composition of the soil microbial community and have implications for our understanding and prediction of microbially mediated processes on minerals, including weathering, soil aggregate formation and organic matter cycling.

Experimental procedures

Experimental design

Avena barbata (Pott ex Link) (wild oat) plants were grown in a soil from a California annual grassland that supports *A. barbata* as a dominant grass species. The soil is a fine-loamy, mixed, active, mesic Typic Haploxeralf (properties described in Supporting Information Table S1) collected from 0–10 cm depth in a pasture at the UC Hopland Research and Extension Centre (38.992938°N, – 123.067714°W) in October of 2014. It was not dried, was sieved to < 2 mm and stored at 4°C until being packed at field density (1.21 g cm^{-3}) into 15 2.5 cm × 15 cm × 30 cm microcosms with a removable side panel (Fig. 1; described in Jaeger *et al*., 1999 and DeAngelis *et al*., 2008a,b). Plants (4 per microcosm – equivalent to field density) were grown from seed in the microcosms under 14 h full spectrum light, at 14%$_{vwc}$ moisture, and 400 ppm CO_2 in a growth chamber, rotating locations at least once a week. Soil moisture was monitored by moisture probes, and pots were watered automatically to maintain the target moisture. After a robust rhizosphere had developed against the removable side panel (1-month-old plants), the pots were opened, the side panel was removed and 10 mineral bags (three each of ferrihydrite, quartz and kaolinite and one of the heavy fraction; description follows) were placed directly on top of the roots and soil in a randomized order (using a different randomized order for each microcosm), covered with additional fresh soil, newly collected in November of 2014 from the same location, and the microcosms were resealed (Fig. 1). After 1, 2 and 2.5 months of mineral incubation (at 2.5 months, the plants were beginning to show signs of senescence), five microcosms were opened and all mineral bags were collected. Soil was separated from coarse roots and passed through a 2 mm sieve to homogenize it and then subsampled and preserved for analysis. All mineral and soil samples were immediately frozen on dry ice and placed in a – 80°C freezer for storage within the day.

Mineral preparation and properties are described in detail in Neurath and colleagues (in preparation) and are

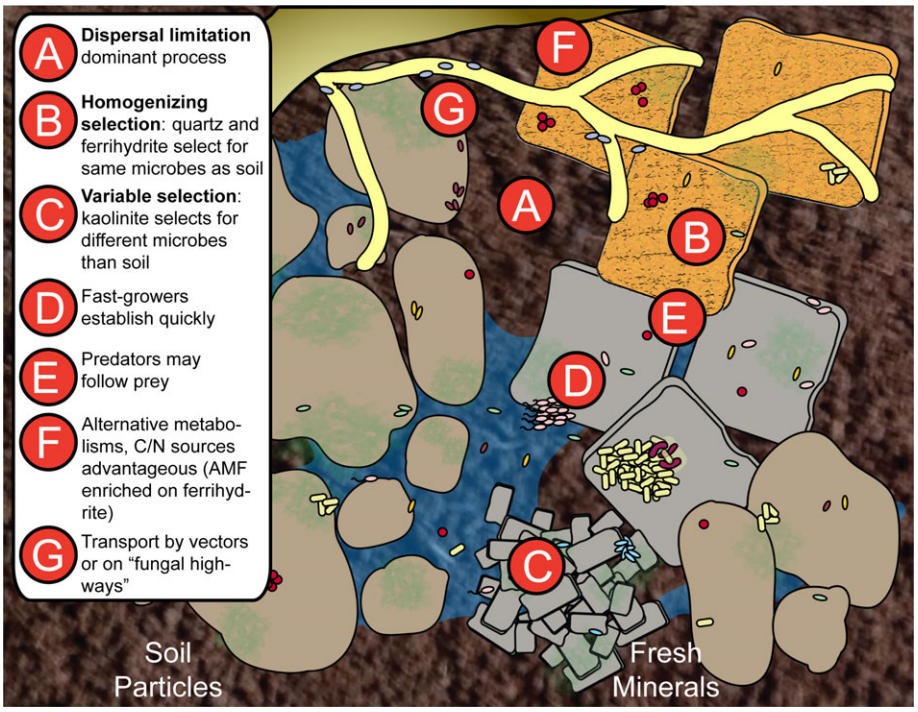

Fig. 7. Conceptual diagram of mechanisms by which microbes may colonize fresh soil mineral surfaces. Dispersal limitation (A) was the dominant process over the timescale of this experiment, while some microbes are likely actively moving or being transported to minerals (G). Once microbes encounter the minerals, homogenizing selection structures quartz and ferrihydrite communities (B), while variable selection structures kaolinite communities (C). Fast growth (D), as predicted by predicted 16S rRNA gene copy number, predation (E) and other advantageous factors (F) may result in specific microbes becoming significantly enriched on the new mineral surfaces, due to a wide variety of possible biological, geochemical and physical drivers. [Color figure can be viewed at wieyonlinelibrary.com]

summarized in Table 1. Briefly, X-ray diffraction (XRD) analysis of soil was used to identify the dominant clay mineral and iron oxide used in this study: kaolinite and ferrihydrite respectively. The soil also contains quartz, which was used as a 'control' mineral due to its low surface area and less-reactive surface. Density fractionation (modified method by Sollins et al. (2006)) was used to separate the 'heavy fraction' (> 1.75 g cm^{-3}) component of soil from free light and occluded light fractions. The heavy fraction was then lyophilized before use in this experiment. Quartz sand was acid washed in 10% HCl. Ferrihydrite-coated quartz was synthesized in the lab, with Al and Si inclusion to better represent a natural ferrihydrite mineral. Kaolinite was mixed with quartz at a 1:1 mass ratio to moderate potential clumping effects of pure clay. Minerals were sealed in 18 μm nylon mesh bags measuring 50 mm × 50 mm × 2 mm. There are numerous challenges in choosing an appropriate mesh size – too small and large-scale interactions are limited; too large and small particles may be lost (Lecerf, 2017). Similar studies have used larger mesh sizes of 50 μm (e.g., Colin *et al*., 2017; Berner *et al*., 2012). In this study, a smaller mesh size of 20 μm was chosen to further limit root access while allowing fungal hyphae in. The thin width of the bags (2 mm) meant that the minerals were within range of what is often defined as 'rhizosphere' soil. Each bag held 6 g of a single mineral type: density-separated heavy fraction; quartz; ferrihydrite-coated quartz ('ferrihydrite') and the 50:50 kaolinite:quartz mix ('kaolinite'). The kaolinite has high surface area, small particle size (< 2 μm) and a relatively low predicted charge density. The quartz has very low surface area, large particle size (210–297 μm) and very low predicted charge density, while the ferrihydrite has moderate surface area, large particle size (210–297 μm) and high predicted charge density. It is important to note the dramatic particle size and surface area differences across the minerals and to recognize that the properties we are considering are, thus, not only chemical properties of these minerals, but also physical properties. We chose not to attempt to make these properties the same across minerals, because they are fundamental properties of how these minerals would appear in soils – e.g., clay mineral particles tend to be very small. The quartz, ferrihydrite and kaolinite minerals had an initial C content of 0%, while the heavy fraction had an initial C content of 1.6%. The minerals were not sterilized, but we were not able to extract sufficient DNA from them to sequence prior to incubations.

DNA extraction

At harvest, minerals and bulk soil samples were transferred to Whirl-pak bags, frozen on dry ice and stored at – 80°C. Anticipating potential difficulty in DNA desorption from the ferrihydrite minerals in particular, we used a sterile cell release buffer for all minerals – Tween20 (5 g l^{-1}) and sodium pyrophosphate decahydrate (1 g l^{-1}) with 0.5 g soil or minerals (Supporting Information Note S3). Released cells were processed using a modified phenol-choloroform DNA extraction protocol (Griffiths *et al*., 2000; Shi *et al*., 2015). Briefly, samples received 500 μl 5% CTAB/0.7 M NaCl/240 mM K-PO_4 at pH 8, 500 μl of 25:24:1 phenol/chloroform/isoamyl alcohol and lysing matrix E beads (MP Biomedicals, Santa Ana, CA). Tubes were shaken on a FastPrep (MP Biomedicals, Santa Ana, CA) for 30 s on speed 5.5. After centrifuging at 4°C, the aqueous phase was transferred to 2 ml phase-lock gel heavy tubes (5 Prime; Quantabio, Beverly, MA), where they received an equal amount of 24:1 chloroform/isoamyl alcohol, were mixed and centrifuged, and then, the aqueous phase was transferred to 1 ml 40% PEG 6000/1.6 M NaCl, where DNA precipitated for 1 h. Extracted samples were then re-extracted with 500 μl CTAB mixture, with the resulting aqueous extract added to the PEG 6000 tubes, along with 1 μl linear acrylamide as a co-extractant. Samples were then centrifuged for 20 min at 4°C, supernatant was removed, then, DNA pellets were rinsed with 70% EtOH, air-dried and resuspended in 50 μl RNAase-free water and frozen at – 80°C. DNA was quantified using a Quant-iT PicoGreen double stranded DNA assay kit (Invitrogen, Carlsbad, CA) and a BioRad iCycler (BioRad Laboratories, Hercules, CA).

Quantitative PCR

The 16S rRNA gene and ITS DNA copies in each sample were determined using quantitative PCR (qPCR) with primer sets EUB338/EUB518 for bacteria and 5.8S/ITS 1f for fungi (Supporting Information Table S2) (Fierer *et al*., 2005), using a BioRad iCycler (BioRad Laboratories) and SSOFast EvaGreen Supermix (BioRad Laboratories). Samples were run in triplicate (10 μl EvaGreen supermix 2×, 1 μl 10 μM f primer, 1 μl 10 μM reverse primer, 1 μl (1:100 diluted or 1:10 diluted, depending on starting DNA concentration) template DNA and 7 μl H_2O; reaction was 95°C for 5 min, [95 °C for 10 s, 62°C for 20 s] ×40. There are important constraints on the interpretation of qPCR data, due to variability in within-taxon copy number and amplicon length, as discussed in Supporting Information Note S1. In light of these issues, we provide qPCR data only for the reader's interest and caution the reader against interpreting differences between treatments.

16S rRNA gene and ITS2 sequencing

We used a two-step PCR to prepare amplicon libraries as described previously (Wu *et al*., 2015). For the first step, primer sets used for amplification of the ITS2 and

16S rRNA genes were gITS7F/ITS4R (fungal ITS2) (White *et al*., 1990) and 515F/808R (bacterial and archaeal 16S rRNA gene v4 region) (Supporting Information Table S2). For the second step, primers with different-length (phased) spacers (to increase base diversity in sample library sequences), barcodes, Illumina sequencing region and Illumina adapters were used. Procedures differed from Wu and colleagues (2015) in amplification cycles (10 cycles in the first step and 20 cycles in the second step for 16S rRNA gene; 12 cycles in the first step and 22 cycles in the second step for ITS2). Sample libraries were sequenced on a MiSeq system (Illumina, San Diego, CA) (2 × 250 bp paired ends) at the Institute for Environmental Genomics, University of Oklahoma.

16S rRNA gene and ITS2 sequence data analysis

For processing and analysing the 16S rRNA gene data, we drew on methods from (McMurdie and Holmes, 2013; Pepe-Ranney *et al*., 2015). We used Paired End reAd mergeR (PEAR) v.0.9.6 (Zhang *et al*., 2014) to merge reads, screed databases (Nolley and Brown, 2015) to demultiplex sequences, cutadapt v.1.8.1 (Martin, 2011) to remove primers, USEARCH v.8.0 (Edgar, 2013) to filter reads and for OTU clustering (97% ID), mothur v.1.35.1 (Schloss *et al*., 2009) for alignment-based quality control and QIIME v.1.8 (Caporaso *et al*., 2010) to assign taxonomy, using the greengenes 97% ID OTU taxonomy database (details in Supporting Information Note S4). For the ITS2 data, we processed them as for the 16S rRNA gene data, drawing on methods from (Bálint *et al*., 2014; Glassman *et al*., 2015), with the addition of using ITSx (Bengtsson-Palme *et al*., 2013) to extract only the fungal ITS2 regions of the reads, and using the UNITE reference database (Kõljalg *et al*., 2013) at 97% ID to assign taxonomy (details in Supporting Information Note S4). We assigned AMF status to any taxa within the *Glomeromycota* phylum, and EMF status to taxa identified as EMF by Glassman and colleagues (2015). These assignments were largely consistent with the results from using the FUNGuild database at the 'highly probable' or 'probable' confidence rankings (Nguyen *et al*., 2016).

Community assembly processes

To determine the dominant processes that may be structuring communities in the minerals and rhizosphere soils, we used the approach described by Stegen and colleagues (2013), where ecological processes are classified into the following categories: (i) homogeneous selection (abiotic or biotic pressures select for the same types of characteristics across communities), (ii) variable selection (abiotic or biotic pressures select for different types of characteristics across communities), (iii) homogenizing dispersal (individuals can move between communities easily), (iv) dispersal limitation (individuals cannot move between communities easily) and (v) undominated (population fluctuations are essentially due to weak selection, weak dispersal and/or random chance events) (Stegen *et al*., 2013, 2015). The process is described in detail in Stegen and colleagues (2013, 2015), but briefly, to follow the process for a given pair of samples, we calculate phylogenetic dissimilarity between the two samples using the β Mean Nearest Taxon Distance (βMNTD), which is the distance between each taxon and its nearest relative in the comparison sample, weighted by its relative abundance and then summed for all OTUs and averaged between the two samples. We then normalize this value and compare it to the expected (null) distribution if no selection were present. The null distribution is calculated by shuffling taxa and their abundances randomly across the phylogeny and then calculating the βMNTD, 999 times. If the community is significantly less similar than expected by null expectations, the actual value will be more than 2 standard deviations above the mean of the null distribution, suggesting homogeneous selection; if the community is significantly more similar than expected, the actual value will be more than 2 standard deviations below the mean and variable selection is suggested. If the actual value is within 2 standard deviations of the mean, then we next consider whether 'dispersal limitation' or 'homogenizing dispersal' may be inferred by using the modified Raup-Crick metric based on Bray–Curtis dissimilarity (RC_{Bray}; Chase *et al*., 2011). Similar to the βMNTD approach, we calculate a null distribution of possible Bray-Curtis dissimilarities between communities, to which we compare the actual value. The null distribution – what community composition would look like if assembly were primarily controlled by drift – is simulated by maintaining the number of samples in which an OTU is present, and its relative abundance across all samples, as well as total OTU richness and number of sequences for each sample, and then assembling 999 possible communities for each sample in each possible pair of samples. If the actual value is significantly lower than expected – communities are more similar than would be expected under drift alone, then dispersal limitation (as well as drift) may be structuring the community. If the actual value is higher than expected – communities are less similar than would be expected under drift alone, then homogenizing dispersal may be structuring the community. In the remaining cases, where neither βMNTD nor RC_{Bray} are significantly different from the null models, other stochastic processes were considered to be the governing processes ('undominated'). The null model analyses were done within each sampling timepoint, separately. (This approach was chosen to control for the natural progression of the soil

community over time and yielded similar results to comparing all timepoints together.) The relative importance of a process was measured as the percentage of comparisons dominated by each process, in all comparisons of a given contrast (e.g., ferrihydrite *versus* soil). We compared communities within all soil samples and between soil and mineral samples for each type of mineral. While the null models were constructed using all the mineral and soil samples for each timepoint, we report the comparisons of each mineral type to soil samples and not between minerals, since we are most interested in the processes that determined how mineral and soil communities were interacting. It is important to remember that this is a predictive statistical model that can determine whether the observed data are consistent with these community assembly mechanisms; it is not a direct experimental test of colonization mechanisms, *per se.* For βMNTD, we used the function 'comdistnt' in the R package picante v.1.6.2 (Kembel *et al*., 2010), and for RC_{Bray}, we used R code from Dr. James Stegen as used in Stegen and colleagues (2013), with additional adjustments for this study, the code for which is included as Supporting Information.

In addition to the βNTI and the RC_{Bray}, we calculated the nearest taxon index (NTI) and net relatedness index (NRI) individually for each sample (Webb *et al*., 2002), with 1000 randomizations, using the picante package v.1.6.2 (Kembel *et al*., 2010) in R (R Core Team, 2011). NRI characterizes the mean phylogenetic distance of taxa in a sample from those in all other samples. NTI characterizes the phylogenetic distance from one taxon to the nearest taxon for each taxon in the sample. NRI is a measure of overall clustering, while NTI is more indicative of terminal clustering (Webb *et al*., 2002).

Statistical analyses

To determine significant differences between minerals and soil for DNA extractions, we performed single-factor ANOVAs and Tukey's HSD in R v.3.3.1 (R Core Team, 2011), log-transforming data to maintain assumptions of normality. To characterize differences in community composition between samples, we performed a non-metric multidimensional scaling (NMDS) analysis on Bray-Curtis dissimilarities between samples, with OTU counts transformed to relative abundance, using the vegan v.2.4–2 package in R (Oksanen *et al*., 2017), with $k = 3$. To determine whether the differences in NMDS plots were significant, we performed a permutational multivariate ANOVA on Bray-Curtis dissimilarities using the vegan package in R (Oksanen *et al*., 2017), controlling for which microcosm each mineral was incubated in where necessary: (relative abundance) ~ (sampling time point) + (mineral type or soil) [+ (microcosm)] (Supporting Information Notes S4 and S5). To determine which taxa significantly increased or decreased in relative abundance in the minerals, compared to in the soil, we used DESeq2 v.1.14.0 (Love *et al*., 2014). We calculated differential abundances for all OTUs for each mineral *versus* the soil for each timepoint (see Supporting Information Notes S6 and S7 for details on outlier OTUs, which DESeq2 excludes based on a Cook's distance identification of outliers, and independent filtering).

Prior work suggests the potential for a bacterial taxon to grow quickly or be a strong early colonizer correlates with 16S rRNA gene copy number (Goldfarb *et al*., 2011; Nemergut *et al*., 2016). To evaluate possible relationships between differential abundances in the minerals *versus* the soils and 16S rRNA gene copy number, we predicted 16S rRNA gene copy number for each OTU using the ribosomal RNA number database (rrnDB-4.4.3) (Lee *et al*., 2009; Stoddard *et al*., 2015). Briefly, we assigned taxonomic names to our OTUs using the Ribosomal Database Project (RDP) database, searched the rrnDB to determine if that genus was included in the database and if it was recorded the mean 16S rRNA gene copy number known for that genus. We note this is only a rough predictor of copy number, since there is known variation even within a single genus, and we were limited by the taxa included in the database, which included only 31% of our OTUs. Thus, our predicted copy number results should be interpreted with caution. To evaluate the relationship between 16S rRNA gene copy number and differential abundance, we built a linear model, using copy number, mineral type and phylum as predictive factors for differential abundance *versus* soil using the *lm* function in the R package 'vegan' (Oksanen *et al*., 2017). We initially included all possible interaction terms, removing those that were not significant for the final model: $\log_2$-fold change ~ (16S rRNA gene copy number) + (mineral type or soil) + (phylum) + (mineral type or soil) $\times$ (phylum).

Acknowledgements

This research is based upon work supported by the U.S. Department of Energy Office of Science, Office of Biological and Environmental Research Genomic Science program under Award Number DE-SC0010570 to UC Berkeley and the University of Oklahoma. Part of this work was performed under the auspices of the U.S. Department of Energy by Lawrence Livermore National Laboratory under contract DE-AC52-07NA27344. The University of California Hopland Research and Extension Center was the source for the soil used here and provided a variety of support services. We thank Katerina Estera, Don Herman, Evan Starr, Shengjing Shi, Erin Nuccio, Anne Kakouridis and Yonatan Sher for field and lab assistance and thank Roland Wilhelm for thoughts on the ecology of *Caulobacter.*

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

Appendix S1. Supplementary information.
Appendix S2. Supplementary Data 1.
Appendix S3. Supplementary Data 2.