Model substrate particles uncover dynamics of microbial communities associated with particulate organic matter decomposition in soil

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Running Title: Model particle associated microbial communities

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Two-part abstract

Abstract

Soil organic matter is the largest terrestrial reservoir of organic carbon. Its particulate fraction, particulate organic matter (POM), serves as a resource and surface for microbial colonization. Degradation of complex biopolymers like cellulose and chitin requires extracellular enzymes produced by phylogenetically diverse microbes. Despite their importance for carbon cycling, the structure and spatio-temporal dynamics of particle-associated microbial communities in soil and how specific substrates influence them remain poorly understood. This study investigated whether microbial communities associated with POM change in composition and richness over time and whether chitin and cellulose select for distinct fungal and bacterial taxa. We incubated self-manufactured millimeter-sized model POM substrate particles containing chitin or cellulose in soil under laboratory and field conditions. Over 50 days, we assessed particle-associated bacterial and fungal communities at multiple time points in the lab and after 47 days in the field. Our results show that community structure and temporal dynamics of particle-associated microbial communities were substrate-specific. While microbial biomass increased on both particle types, chitin-associated microbial exhibited stronger temporal changes. Communities on chitin and cellulose particles were enriched in specific bacterial and fungal genera compared to communities in the surrounding soil. In conclusion, our study demonstrates that microbial communities associated with model chitin particles underwent notable temporal changes. More specifically, microbial richness decreased, and community composition shifted over the incubation period. Both chitin and cellulose particles favored certain fungal, and especially bacterial genera compared to surrounding soil.

Importance

Particulate organic matter constitutes the largest terrestrial organic carbon storage. Microorganisms colonize, degrade, transform, and become part of organic matter. We use self-manufactured model chitin or cellulose particles to examine the temporal dynamics of bacterial and fungal communities associated with particulate organic matter in soil. Our findings suggest model particles can be used to investigate particle-associated microbial communities in soil. Furthermore, we show that temporal dynamics are substrate-specific. Our findings advance our understanding of particle-associated microbial communities and the impact of specific substrates on microorganisms in soil.

Keywords

Soil microbial communities, particulate organic matter, model particles, chitin, cellulose, temporal dynamics

Introduction

Soil organic matter is the largest terrestrial carbon reservoir (Lehmann and Kleber, 2015; Angst et al., 2021). Conceptually, soil organic matter can be divided into mineral-associated organic matter and particulate organic matter (Lavallee et al., 2020). Particulate organic matter serves as a nutrient source to microorganisms and a surface for colonization (Young et al., 2008). Particles are considered hotspots for microbial activity in soil (Witzgall et al., 2021) as they represent spatially isolated, nutrient-rich microhabitats in the otherwise nutrient-poor soil environment.

Microbes decompose particulate organic matter, as they can only take up small molecules (typically less than 600 Da) across their cell walls (Arnosti et al., 2014; Lehmann and Kleber, 2015). To acquire resources, they produce and excrete extracellular enzymes into their surroundings (Allison et al., 2010; Nunan et al., 2020). Extracellular enzymes are the agents of organic matter decomposition, as they break down large molecules into subunits and ultimately monomers, which can be taken up by microbes (Sinsabaugh et al., 2008; Gómez et al., 2020). By decomposing soil organic matter via extracellular enzymes, heterotrophic microorganisms mediate the recycling of carbon and nutrients (Sinsabaugh et al., 2008; Bardgett and Van Der Putten, 2014; Berlemont, 2017; Wu et al., 2024). Extracellular enzymes are substrate specific (Allison et al., 2010) and metabolically costly to produce (Nunan et al., 2020, Guseva et al., 2024). The complete breakdown of biopolymers requires multiple enzymes that act in synergy (Sinsabaugh et al., 2009; Talamantes et al., 2016, Guseva et al., 2024). Genetic potential to produce particular extracellular enzymes differs between microbial taxa (Berlemont and Martiny, 2013, 2015; Zimmerman et al., 2013; Arnosti et al., 2014). Consequently, phylogenetically diverse microbial communities are necessary to carry out all steps of biopolymer degradation.

Cellulose and chitin are the two most abundant natural polymers in nature (Das et al., 2010; Berlemont and Martiny, 2015). Cellulose is the major component of plant material and the most abundant biopolymer in nature, and chitin is the second most abundant polysaccharide (Das et al., 2010; Wieczorek et al., 2014). Both need to be enzymatically degraded prior to microbial uptake. While the potential to degrade these polysaccharides is widespread across bacterial taxa, the structure of degrader communities has been found to be driven by environmental conditions and substrate availability in situ (Berlemont and Martiny, 2015; D. Li et al., 2023). For instance, the ability to degrade cellulose has been shown to be more widespread amongst fungi than their ability to degrade chitin (Berlemont, 2017).

Spatial proximity of microbial cells to enzymatically degraded substrates is advantageous to enhance the return of enzyme investment. Microbial attachment to particulate organic matter and formation of spatially structured communities should theoretically minimize losses of

enzymatic breakdown products to the surrounding environment through diffusion. The presence of both, bacteria and fungi, has been detected on particulate organic matter in soil. More specifically, it has been shown that bacteria occur in biofilms on the surface of particulate organic matter and that fungal hyphae pervade bacterial biofilms (Witzgall et al., 2021). However, overall, little is known about microbial communities associated with particulate organic matter in soil, despite the relevance of particulate organic matter-degrading communities for carbon and nutrient cycling. We lack knowledge about the assembly and temporal dynamics of microbial communities on particulate organic matter, and whether specific substrates select distinct microbial communities and particular microbial taxa in soil.

This lack of knowledge is at least partly due to the complex nature of soil. It is challenging to study microbial communities associated with particulate organic matter in soil. Soils are opaque and characterized by high substrate heterogeneity (Young et al., 2008). Organic matter exhibits diverse chemical composition and is spatially heterogeneously distributed throughout the soil matrix (Lehmann et al., 2008, 2020; Cotrufo et al., 2013; Lavallee et al., 2020; Schlüter et al., 2022). Moreover, the extraction of particulate organic matter from soils requires chemical agents that potentially alter associated microbial communities and distort research findings. Investigating microbial communities of native particulate organic matter that have been chemically extracted from soils might lead to erroneous results.

In this study, we developed and carried out an experimental approach for studying microbial communities associated with particulate organic matter in soil. More specifically, we employed self-manufactured model substrate particles which either contained chitin or cellulose. Model particles mimic natural particulate organic matter, with the advantage that they contain one specific, known polymer. Model substrate particles have been used to study microbial community assembly and dynamics on particles in aquatic systems (Cordero and Datta, 2016; Datta et al., 2016; Ebrahimi et al., 2019), but, to our knowledge, not in soils. We incubated model substrate particles in sieved forest soil in the lab and the field and examined microbial communities of model particles and the soil in which the particles had been incubated, at various time points over a 50-day-long incubation in the lab and after 47 days of incubation in the field. Using this approach, we examined (i) whether model substrate particles select for specific subsets of the overall soil community, (ii) whether particle-associated microbial communities undergo temporal changes, and (iii) whether chitin and cellulose select for specific bacterial, archaeal, and fungal communities and particular microbial genera. We amplicon sequenced bacterial, archaeal, and fungal communities of model substrate particles and the soil in which the particles had been incubated and assessed their taxa richness and community composition. In addition, we determined microbial biomass on particles and in soil via digital droplet PCR.

Material and Methods

Model particle production

We manufactured three types of model particles in the lab: (i) model particles containing chitin (“model chitin particles”), (ii) model particles containing cellulose (“model cellulose particles”), and (iii) model particles containing neither chitin nor cellulose (“”no substrate” model particles”). Model particles were designed to mimic particulate organic matter. Model particles to which neither chitin nor cellulose was added served as controls for the chosen scaffold material of model particles, gellan gum. We used Gelrite® gellan gum as a gelling agent for the model particles because of its physical properties (e.g., rapid and stable gelling in the presence of ionic cross-linkers, high transparency for visualization) and its resistance to enzymatic degradation, which makes it a stable and relatively biologically inert scaffold for embedding the selected substrates (chitin and cellulose) (Das et al., 2015). Gellan gum is biologically rather inert compared to other frequently used gelling agents, alginate and agarose (Wang et al., 2023). For model particle production, we followed a protocol by Mußmann (Mußmann et al., in prep). In detail, we dissolved 1.5 g of Gelrite gellan gum (Carl Roth) in 100 mL of pure MilliQ water in a beaker that was placed on a hotplate magnetic stirrer to ensure dissolution of gellan gum. We added a magnetic stirring stick to the beaker to ensure constant mixing of the solution. To facilitate polymer cross-linking and stability, 0.117 g 8 µM $CaCl_2$ was added. Additionally, we added 5 mL of 10 % w/v magnetite solution to the mixture to give particles a magnetic property. The magnetic feature of model particles facilitated the separation of particles from liquids in washing steps further downstream. We used approximately 1.4 g of chitin from shrimp shells (Sigma-Aldrich) or cellulose powder (Sigma) per plate. Produced model particles were of cylindric shape and were approximately 3 mm heigh and 2 mm wide. Model particles were UV-sterilized and stored in 20% ethanol at 4°C until use. For details of particle production see Mußmann et al. (submitted).

Soil collection and processing

Soil was collected in a beech-dominated temperate forest near Klausen - Leopoldsdorf, Austria (48.11925 N, 16.04343 E, 580 m altitude) in spring (April 26, 2021). The litter layer was removed prior to soil sampling. Soil cores were taken from the upper five centimeters of mineral soil at five previously established 5 x 5 m field plots (Simon et al., 2024) using a 5 cm x 8 cm metal corer. For each plot, two soil cores were pooled and sieved using a 2-millimetre-mesh sieve and homogenized into a single representative sample for each plot, producing five composite soil samples.

Set-up of laboratory and field incubation

We incubated model particles in sieved forest soil in the laboratory and in the field. For the lab incubation, we handcrafted mesocosms (3 cm in diameter and 5 cm in height) using 50 mL plastic Greiner vials. Each vial was cut at 5 cm height (30 mL mark on the side of the vial) and the part of the vial with the screw cap attached served as the mesocosm, the cap being the bottom of the mesocosm. For the field incubation, we sewed mesh bags from screen printing fabric (100% polyester, 120 threads per cm) with a mesh width of 40 µm, which allows the ingrowth of fungal hyphae but excludes the ingrowth of roots.

We set up four incubation treatments: i) 2-mm-sieved soil plus model chitin particles, ii) 2-mm-sieved soil plus model cellulose particles, iii) 2-mm-sieved soil plus model particles containing neither chitin nor cellulose, and iv) 2-mm-sieved soil without model particles (Fig. 1). We rinsed model particles, which had been stored in 20% ethanol, three times in 15 mL pure MilliQ water to remove residual ethanol. We added 1 g of model particles to 15 g of sieved soil to individual plastic bags and shook them to homogenize and distribute model particles evenly throughout the soil. Moreover, 15 g sieved soil which was incubated without model particles was shaken to guarantee proper homogenization. Soil (and model particles) was filled in lab mesocosms and field mesh bags (on April 28, 2021). Soil in mesocosms was compacted to resemble field density using a 50 mL syringe plunger. Mesocosms were covered with parafilm during incubation to prevent desiccation and they were weighed weekly to check and potentially adjust moisture content if necessary. Full mesh bags were sealed with a heat sealer and measured 7 cm x 10 cm in size. In total we set up 80 mesocosms and 20 mesh bags (Fig. 1).

Mesocosms were incubated at 11°C, matching the soil temperature in 5 centimeters soil depth measured on the day of soil sampling (April 26, 2021. Filled mesh bags were kept in closed plastic bags at 11°C in a lab incubator between setting them up and burying them in the field. Mesh bags were buried in the field at a depth of 5 cm (April 30, 2021).

Destructive harvest

Lab mesocosms were destructively harvested 1, 13, 29 and 50 days after the start of the lab incubation (start of incubation on April 28, 2021, destructive harvest on April 29, May 11, May 27, June 17, 2021). Field mesh bags were destructively harvested after 47 days of incubation in the field (filling of mesh bags on April 28, 2021, buried on April 30, 2021, destructive harvest on June 14, 2021). Upon destructive harvest, we pushed soil cores out of mesocosms and separated the model particles from soil by hand using tweezers. We washed the model particles to get rid of soil sticking to the particles. We submerged model particles in MilliQ water in a petri dish, carefully swayed petri dishes by hand, and discarded

liquid while holding back the particles with a strong magnet. We repeated the washing steps three times to obtain the particles and the directly attached microbial communities for subsequent analysis. The magnetic feature of particles facilitated the separation of model particles from the “dirty” liquid. Aliquots of washed model particles were (i) weighed for DNA extraction and (ii) fixed with ethanol for downstream microscopic analysis.

DNA extraction and sequencing

DNA extraction, library preparation, and amplicon sequencing were conducted as in Simon et al 2024. In short, we extracted DNA from a minimum of 20 model particles (150 mg ± 0.04) and 250 mg soil (253 mg ± 0.005) with the Qiagen PowerSoil kit (Qiagen, Valencia, CA, USA). Except for adding a bead beating step (30 sec), we followed the manufacturer´s protocol. For amplicon sequencing, we followed a two-step PCR barcoding approach (Pjevac et al., 2021) to amplify the V4 region of the 16S rRNA genes targeting bacteria and archaea, and the fungal internal transcribed spacer region 2 (ITS2) of the nuclear rRNA operon. We used primers 515F (Parada et al., 2016) and 806R (Apprill et al., 2015) for 16S rRNA genes. For the ITS2 region, a nested approach (Nilsson et al., 2019) was chosen, generating a PCR product using primers ITSOF-T (Taylor and McCormick, 2008; Tedersoo et al., 2008) and ITS4 (White et al., 1990), which was then used as a template for a second PCR using primers gITS7 (Ihrmark et al., 2012) and ITS4. Amplicons were sequenced on a MiSeq platform (Illumina, V3 chemistry, 600 cycles) at the Joint Microbiome Facility (JMF) of the Medical University of Vienna and the University of Vienna under project number JMF-2106-12. Raw data processing was performed as described previously (Pjevac et al., 2021).

Digital droplet PCR (ddPCR)

We quantified the number of copies of the V4 hypervariable region of the 16S rRNA gene and of the fungal ITS region of model particles and in soils with digital droplet PCR (ddPCR). Gene copy numbers can be used as a proxy for microbial biomass (Alteio et al., 2021). Prior to performing ddPCR, we measured DNA concentration of all DNA extracts using PicoGreen (Quant-iT PicoGreen dsDNA Assay Kit; Thermo Fisher Scientific). If the concentration was under the detection limit, we re-measured extracts using a Qubit high-sensitivity dsDNA assay (Thermo Fisher Scientific). For 16S ddPCR, DNA extracts were diluted to a concentration of 0.05 ng/µL and for ITS ddPCR to a concentration of 0.25 ng/µL. 2 µL of diluted DNA template were used as input for each ddPCR assay. Gene copy numbers per µL DNA extract were assessed with the QX200 Droplet Digital PCR (Bio-Rad) and analyzed with the QuantaSoft software (Bio-Rad).

Particle fixation and subsequent fluorescence microscopy

A fraction of model particles (~ 12 model particles) which had been incubated were fixed with 96% ethanol in 2 mL Eppendorf tubes. Tubes were stored at 4 °C overnight. Supernatant was removed using a magnetic pulldown and model particles were resuspended in 1.5 mL of 1X PBS. These washing steps were repeated three additional times. Ethanol-fixed samples were suspended in 1.5 mL of cold 96% ethanol. Model particles were inverted to mix. Following fixation, samples were stored at -20 °C. DNA associated with model particles was stained with the stain SYBR Green I nucleic acid stain (Invitrogen, Thermo Fisher). Selected chitin particles stained with SYBR Green were analyzed using a Leica confocal laser scanning microscope, coupled with a Thunder imaging system (Leica).

Data analyses

Amplicon sequence variants (ASVs) were inferred using the DADA2 R package v1.20 (https://www.ncbi.nlm.nih.gov/pubmed/27214047) applying the recommended workflow (https://f1000research.com/articles/5-1492). 16S rRNA FASTQ reads 1 and 2 were trimmed at 220 nucleotides (nt) and 150 nt respectively with allowed expected errors of 2. ITS FASTQ reads 1 and 2 were trimmed at 230nt with allowed expected errors of 2 and 4 respectively.

16S rRNA ASV sequences were subsequently classified using DADA2 against the SILVA database SSU Ref NR 99 release 138.1 (https://doi.org/10.5281/zenodo.4587955, https://www.ncbi.nlm.nih.gov/pubmed/23193283) using a confidence threshold of 0.5. ITS ASV sequences were classified against the UNITE all eukaryotes general FASTA release version 8.2 (Abarenkov et al, 2020) using a confidence threshold of 0.5.

All data analyses were performed in R version 4.4.0 (R Core Team, 2021). We analyzed samples that had yielded at least 3000 sequencing reads (16S rRNA genes: n = 172, ITS2 sequences: n = 179, see Table S1 and S2 for numbers of samples per treatment and time point).

To compare the number of bacterial, archaeal, and fungal amplicon sequence variants (ASVs) detected in the model particle fractions and in soil, we randomly rarefied samples to 3015 (16S rRNA genes) and 5676 (ITS2 sequences) reads to establish equal sequencing depth across samples. To determine whether model substrate particles favored microbial genera compared to the soil source community, we performed differential abundance analysis based on relative abundance data using the R package ANCOMBC (v. 2.6.0) (Lin and Peddada, 2020). Using the analysis of compositions of microbiomes with bias correction (ANCOMBC), we determined genera which were significantly enriched and depleted in microbial communities associated with model particles compared to the soil, in which particles had been incubated (“surrounding soil”). In this study, we focus on the genera that

were significantly enriched on model particles compared to the “surrounding soil”. In addition, we examined temporal dynamics of particle-associated microbial communities by visualizing changes of microbial community composition over the incubation period with principal coordinate analysis (PCoA) plots. PCoA plots visualize Aitchison distances, Euclidean distances calculated from centered-log-ratio transformed counts matrices, which account for the compositional nature of amplicon sequencing data (Gloor et al., 2017).

Results

Microbial biomass increased on model particles over time, but not in the surrounding soil

We assessed microbial colonization and biomass of model particles using both droplet digital PCR (ddPCR) and microscopic evaluation. Bacterial and archaeal, and fungal biomass (significantly) increased on model particles over time, as evidenced by increasing gene copy numbers on model chitin and cellulose particles over the 50 days and 47 days incubation period for the lab mesocosms and the litter bags incubated in the field, respectively (Fig. 2A). The number of 16S rRNA gene copies increased most on model chitin particles, from 45.13±24.68 million gene copies per gram particles at the first time point to 328.61±163.32 million after 50 and 262.19±370.49 million after 47 incubation days, respectively. Bacterial and archaeal gene copy numbers also slightly increased on “no substrate” model particles, however not significantly (Fig. 2A). In contrast, fungal ITS copy numbers differed significantly between particles after one day of incubation and 29 or 50 days of incubation (Fig. 2B). At most time points, gene copy numbers were similarly small or large for model cellulose particles and “no substrate” model particles, but larger on model chitin particles (Fig. 2A, 2B). Particles incubated in the lab and the field were similarly densely populated by microbes after 47 and 50 days. In contrast, microbial biomass in the soil surrounding the model particles, as well as soil incubated without additional substrate did not reveal a clear pattern over time (Fig. 2C, 2D). Microscopy visualization of chitin particles supports the finding that particles were more densely populated after several days of incubation than after one day (Fig. 2E).

ASV richness of model chitin particles decreased over the incubation period

To determine whether changes in microbial community structure occurred on model particles and in soil across the different treatments, we investigated amplicon sequence variant (ASV) richness of rarefied data. Overall, the richness of initial particle-associated microbial communities was higher compared to communities associated with particles later in the incubation period (Fig. 3). The number of bacterial and archaeal taxa associated with model chitin particles dropped from 1583 ± 135 (median ± standard deviation) on the first day after the incubation start to 908 ± 169 rarefied ASVs at the end of the incubation period (day 50,

Fig. 3A). Also, fungal richness of model chitin particles was lower after multiple days of incubation (332 ± 56.7 at day 50) than after one day of incubation (368 ± 44.8). Bacterial and archaeal, and fungal ASV richness detected on cellulose particles was slightly increased after 13, 29 and 50 days of incubation (16S rRNA: 13d: 1541±157, 29d: 1521±83.5, 50d: 1309±62.9; ITS2: 13d: 476±76.1, 29d: 498±77.3, 50d: 418±77) compared to particles after one day of incubation (16S rRNA genes: 1289±263 ASVs, ITS2: 277±88.5). Bacterial and archaeal ASV richness of “no substrate” model particles slightly decreased from 1566±234 (1d) to 1109±82.7 ASVs (50d), whereas fungal richness clearly increased over the incubation time (260±34 to 365±54.2 ASVs). In contrast to the substrate particles, microbial richness remained relatively more constant across time points and treatments (Fig. 3).

Chitin particle-attached microbial communities underwent temporal changes

Additionally, we investigated whether microbial community structure on model particles and the surrounding soil changed over the incubation period (Fig. 4). Differences in bacterial, archaeal, and fungal community composition between the time points were most pronounced for communities associated with model chitin particles (Fig. 4A, 4D). Communities that were detected on the first day of incubation were most similar to the communities of the soil which was used to set up lab mesocosms. Later communities (after more than one day of incubation (13, 29, and 50 days)) were further apart and hence differed more strongly from initial communities than communities after one incubation day. We found that bacterial and archaeal, and fungal communities that were associated with chitin particles, which had been incubated for 47 or 50 days in the field and the lab, were very similar in their composition (Fig. 4A, 4D). In comparison to model chitin particles, microbial communities associated with model cellulose particles did not show pronounced changes over the course of the incubation period (Fig. 4B, 4E). Bacterial and archaeal communities associated with “no substrate” model particles, the control particles that contained neither chitin nor cellulose, exhibited a similar, yet less pronounced temporal pattern as chitin particles (Fig. 4C). Bacterial and archaeal communities noticeably differed in their composition between “no substrate” model particles that were harvested after one day of incubation from particles that were harvested after multiple days of incubation (Fig. 4C). In comparison, fungal communities associated with control model particles (NS) did not show such a clear separation of the initial soil-communities and the communities associated with particles that were harvested after one incubation day from the communities of particles that were incubated for multiple days (Fig. 4F). Over all treatments, communities in soil did not exhibit strong changes over the incubation period. Composition of communities in soil was rather similar across the different harvest timepoints (1d, 13d, 29d, 50d, 47d) (Fig. 4G-4L).

Certain microbial genera were enriched on chitin and cellulose (particles)

Next, we compared relative abundances of microbial genera between the model substrate particles and the surrounding soil, which was used as the “baseline”, to address whether substrates led to the enrichment of microbial genera on substrate particles using the differential abundance analysis R package ANCOMBC. In this study, we focused on genera which were significantly enriched in their relative abundances on model substrate particles compared to the soil (Fig. 5, 6). We did not only detect genera that were not enriched on model particles, but also genera that were significantly depleted on model particles compared to the surrounding soil (Fig. S1-S7). We observed that certain bacterial and fungal genera were significantly more abundant on model chitin and cellulose particles than in the soil in which these particles had been incubated (Fig. 5, 6). At the same time, other genera were significantly depleted in their relative abundances on model chitin and cellulose particles compared to soil (Fig. S1-S7). In the initial phase of colonization of particles (after one incubation day), few bacterial genera, *Paenibacillus* and *Bacillus* on chitin particles, and KD3-10, *Paenibacillus*, *Clostridium sensu stricto* 1, and *Micropepsis* on cellulose, were relatively significantly more abundant on model particles compared to the surrounding soil (Fig. 5A, 5B). *Asticcacaulis* and *Mucilaginibacter* were the two genera which were enriched on both model chitin and cellulose particles at all later harvest timepoints (13d, 29d, 50d, 47d) with the exception that *Asticaccaulis* was not detected on cellulose particles after 13 incubation days (Fig. 5). Most genera were observed amongst enriched genera only on one model substrate particle type or at certain time points. On chitin particles, bacterial genera *Alkanindiges*, *Silvimonas, Paucibacter, Roseatales,* and *Sphaerotilus* were amongst the most enriched genera at the later time points of the lab incubation (13d, 29d, 50d) (Fig. 5A). We show that a larger number of bacterial genera was significantly increased in their relative abundance on model chitin particles compared to soil than in the case of cellulose particles (Fig. 5). While the number of bacterial genera that were enriched on model chitin particles was similar for lab and field incubations after 47 and 50 days of incubation (Fig. 5A), more bacterial genera were enriched on cellulose particles that had been incubated in the field than in the lab (Fig. 5B). Most bacterial genera, whose relative abundances were significantly increased on particles compared to soil, matched between chitin and cellulose particles of field incubations (Fig. 5A, 5B).

We also found model substrate particles to be enriched in certain fungal genera compared to the surrounding soil (Fig. 6). On both model chitin and cellulose particles, *Trichoderma* was significantly relatively more abundant than in soil after one incubation day (Fig. 6A, 5B). Fungal genera that were significantly enriched on chitin particles differed among the harvest timepoints (Fig. 6A). *Pseudogymnascus* was relatively more abundant on cellulose particles than in soil after 13 and 29 days of lab and 47 days of field incubation (Fig. 6B). Cellulose

particles were also significantly enriched in *Ilyonectria* compared to corresponding soil after one, 13 and 50 days (Fig. 6B). Overall, significant differences in genera between model chitin particles and the soil were less pronounced for fungi than for bacteria (Fig. 6A, 5A). Considerably fewer fungal than bacterial genera were significantly different in their relative abundances between model substrate particles and the soil (Fig. 6, 5).

Discussion

Our work indicates that microbes that were detected on model particles were physically attached to these particles, as they would otherwise have been removed during the washing steps. These findings go in line with a previous study that proposed that bacteria and archaea are widely surface-attached in soil as the energetic costs of motility in soils are high (Nunan et al., 2020). At the same time, our study suggests that many bacterial and archaeal taxa were able to move towards particles and attach to them within one day of particle incubation in soil. It is likely that colonization of particles did not occur solely through adjacent soil surfaces but rather that bacteria actively or passively moved to substrate particles. The fast arrival of a large proportion of the microbial source community suggests that motility is widespread among bacteria, archaea, and fungi in the here investigated forest soil. The increase in microbial biomass associated with model substrate particles indicates either continuous arrival of microbes on the particle surface or growth of already attached microbes. Microbial growth would then be fostered by the degradation of chitin and cellulose via extracellular enzymes which make resources available for microbial growth. It is possible that a certain fraction of model particle-associated microbes was lost due to the employed washing steps, which we cannot account for.

Analysis of changes in microbial richness revealed a decrease in bacterial, archaeal, and fungal richness on model chitin particles over time. In contrast, bacterial and archaeal richness remained unchanged on cellulose particles and “no substrate” particles, which contained no additional substrate, during the incubation period and fungal richness even increased on both model particle types. Bacterial, archaeal and fungal community composition analysis showed that microbial communities associated with chitin particles underwent temporal changes in community composition, whereas cellulose particles did not exhibit clear temporal patterns. These findings indicate that chitin more strongly selects for substrate-specific microbial consortia than cellulose. Communities associated with chitin particles seemed to become more chitin-specific over time, which points towards microbial communities undergoing temporal succession on particulate organic matter containing chitin as has been shown by others (Enke et al., 2018). In contrast, cellulose seemingly did not select for specific microbial communities as strongly as chitin. This might be a consequence of the different chemical complexity of chitin, necessitating a specific suite of enzymes or

microbial interactions to be degraded. In contrast to model chitin particles, we did not detect temporal patterns of microbial community composition for the soil, in which model chitin particles had been incubated. Our results demonstrate that we might not be able to study the effect of complex particulate substrates on microbial communities with composite soil samples. Our results demonstrate that millimeter-sized model particles are a suitable study system for investigating substrate-specific microbial communities in soil and their temporal dynamics.

Fewer bacterial genera were enriched on model cellulose particles than on model chitin particles in the lab incubation. This finding supports our claim that chitin exerts a stronger selective pressure on microbial communities than cellulose does. The bacterial and fungal genera that were enriched on model chitin and cellulose particles compared to the soil metacommunity potentially were particularly well adapted to utilize or degrade chitin, cellulose or their degradation by-products. Members of some bacterial and fungal genera that were enriched on model substrate particles have been described to possess the genetic potential to produce chitin or cellulose-degrading extracellular enzymes. After one incubation day, chitin particles were enriched in the bacterial genus *Paenibacillus* and *Bacillus*, bacterial genera which include known chitin degraders (Watanabe et al., 1990; Itoh et al., 2013). Cellulose particles were also enriched in *Paenibacillus* as well as *Micropepsis* and *Clostridium sensu stricto* after one incubation day, representing genera which contain known degraders of cellulose or cellobiose, the repeating unit of cellulose (Harbison et al., 2017; Kim et al., 2018; Yadav and Dubey, 2018; C.-J. Li et al., 2023). The bacterial genus *Paenibacillus* was also significantly enriched on “no substrate” particles, which could indicate that members of this genus are especially efficient in colonization of newly introduced particulates to soil, irrespective of the specific substrate contained. The bacterial genus *Asticcacaulis* (phylum Alphaproteobacteria) has been found to contain many sequences for glycoside hydrolases, key enzymes targeting both cellulose and chitin, thereby supporting its role as a generalist with regard to polymeric carbon degradation (Talamantes et al., 2016). Members of *Mucilaginibacter* have been described to also possess the potential to produce certain glycoside hydrolases and be capable of degrading chitin and cellulose (Wang et al., 2020; Kim et al., 2022). Species of other significantly enriched genera on chitin and/or cellulose particles, *Silvimonas* and *Collimonas,* have been found to possess chitinases (Yang et al., 2005; Song et al., 2015, 2015; Ballhausen et al., 2016), and a member of *Roseateles* was found to degrade cellulose (So et al., 2024). However, not all taxa detected on model substrate particles were necessarily degrading the substrate themselves. Some taxa might profit from the degradation products of enzymes produced by other microbes that are present, or they might feed on metabolites of degraders. Additionally, it is important to note that degradation potential for microorganisms is not necessarily conserved at the genus

level, and our ability to assign specific taxa to degradation of a specific substrate is limited based on amplicon sequencing results. It would be advantageous if future studies would investigate genes and extracellular enzyme activity of taxa which are associated with model substrate particles to uncover taxa that degrade specific substrates.

Only a small number of fungal genera significantly increased in relative abundance on model chitin and cellulose substrate particles compared to the surrounding soil. The ability to degrade chitin or cellulose potentially is more widespread among fungal taxa than bacterial taxa (Rytioja et al., 2014; Berlemont, 2017). Consequently, these specific substrates might exhibit a lower selective pressure on fungi than on bacteria. Comparison of model particles, which did not contain chitin nor cellulose, with the surrounding soil, shows that differences in relative abundances of microbial genera between model substrate particles and the soil were driven by the specific substrate and not the compounds used to produce model particles.

Field incubations showed different patterns of enrichment of microbial genera on model substrate particles than lab incubations. Whereas more bacterial genera were enriched on model chitin particles than on model cellulose particles in the lab, numbers were equal for model substrate particles that had been incubated in the field. Moreover, more fungal genera were enriched on model chitin and cellulose particles that were incubated in the field than in the lab. For bacteria and fungi log fold changes were larger for field-incubated particles than for lab-incubated particles. Differences in research findings can be expected between field and lab incubations. Conditions in the lab are relatively more stable than in the field. In this study, mesocosms containing model particles and soil were incubated at constant 11°C. In contrast, the content of mesh bags that were buried in the field were exposed to temporal fluctuations in soil temperature and soil moisture over the incubation period. Moreover, the potential impact of plant exudates on microbial communities in the field is absent in the lab. The here found differences between lab and field incubations underpin that caution must be taken when translating findings of lab studies to nature.

Our study shows that model substrate particles are a suitable tool for studying substrate-specific microbial communities on particulate organic matter in soil and their temporal dynamics. To the best of our knowledge, model particles have been used to study particle-associated microbial degrader communities in aquatic ecosystems (Cordero and Datta, 2016; Datta et al., 2016; Ebrahimi et al., 2019), however our study is the first to investigate microbial communities associated with particulate organic matter in soil.

Our study underpins that self-manufactured model substrate particles provide a highly flexible, standardized experimental system for the investigation of microbial degrader communities (Mußmann et al., in prep, Cordero and Stocker, 2017; Enke et al., 2019). One can choose the size and the shape of particles and add the substrate of interest to the

mixture, in particulate (Mußmann et al., in prep). Particles can be investigated under the microscope due to the high transparency of the used gelling agent, Gelrite gellan gum. A caveat of the here employed recipe for model particles may be the use of a carbon-containing gelling agent as it could potentially be metabolized by microbes. It has been shown that some microbes are able to degrade gellan gum (Das et al., 2015). However, all polymers which could be used as an embedding matrix for substrates may be degraded by some microorganisms and thus support microbial growth of some microorganisms. The low increase in gene copies on gellan gum particles indicates that these particles were unspecifically colonized by microbes. In contrast to the insignificant changes observed in this study, Mußmann et al. (in prep) found significant but also substantially less growth on “no substrate” particles compared to chitin and cellulose particles. Microbial biomass was overall lower on “no substrate” model particles than on model cellulose particles and much lower than on chitin particles at all time points. Moreover, our results suggest that the significant enrichment of genera on model chitin and cellulose particles compared to the surrounding soil was not the result of gellan gum, but a result of the presence of chitin or cellulose. Only few genera including *Paenibacillus*, *Alkanindiges, Trichoderma,* and *Ilyonectria* were enriched in their relative abundances on model chitin, cellulose, and “no substrate” particles. Most genera that were enriched on model chitin and cellulose particles were not enriched on “no substrate” particle controls. Especially after multiple days of incubation in the lab, we did not find any fungal genera to be enriched on “no substrate” particles compared to the soil. In addition, log fold changes of genera were much larger for chitin particles than for “no substrate” particles. Nevertheless, we recommend including “no substrate” model particles as controls for model particles which contain a specific substrate in all experiments. Also, it might be advisable to shorten the here applied incubation duration in future experiments to capture and study more fine-scale temporal dynamics of microbial communities associated with particulate organic matter in soil.

Conclusion

This study demonstrates the value of lab-manufactured model substrate particles as a tool for investigating substrate-specific microbial communities associated with particulate organic matter and their temporal dynamics in soil. Our study demonstrates that complex substrates like chitin or cellulose select for specific microbial communities of the soil microbiome. Microbial communities associated with chitin undergo more strong temporal changes than cellulose, demonstrating the specificity of microbial communities for substrates in soil. Future studies could further advance our understanding which microbial taxa are able to degrade complex biopolymers in soil by employing model substrate particles to isolate and characterize substrate-specific microbial communities.

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Credit authorship contribution statement

Eva Simon: Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. **Lauren Alteio**: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Writing – original draft, Writing – review & editing. **Alexander König:** Investigation, Writing – review & editing. **Bruna Imai**: Investigation, Writing – review & editing. **Julia Horak:** Investigation, Writing – review & editing. **Julia Wiesenbauer**: Investigation, Writing – review & editing. **Joana Séneca**: Data curation, Writing – review & editing. **Bela Hausmann**: Data curation, Writing – review & editing. **Marc Mußmann**: Methodology, Writing – review & editing. **Barbara Kitzler:** Resources, Writing – review & editing. **Christina Kaiser**: Conceptualization, Funding acquisition, Project administration, Supervision, Writing – review & editing.

Conflicts of Interest

The authors declare no conflict of interest.

Data Availability

Raw 16S rRNA genes and ITS2 amplicon sequencing data have been deposited in the NCBI Sequence Read Archive under the BioProject accession number PRJNA1208268. Bacterial, archaeal, and fungal gene copy numbers are available on the Zenodo repository (10.5281/zenodo.14614129).

Figure captions

Fig. 1 Schematic of the experimental set-up. (**A**) We set up four incubation treatments, incubating (i) self-manufactured model chitin (CH), (ii) cellulose (CE), (iii) “no substrate” (NS) particles and (iv) no model particles in 2-mm-sieved soil. We investigated five biological replicates by using soil sampled from five field plots. We set up four technical replicates per treatment and plot for the lab incubation, which we harvested at four time points, after one, 13, 29 or 50 days of incubation in the laboratory, and one technical replicate per treatment and plot for incubation in the field which we harvested after 47 days. (**B**) We mixed 1 g of model particles and 15 g 2-mm-sieved soil and filled it in lab mesocosms that were produced from 50 mL Greiner tubes or mesh bags for the field. Upon harvest of mesocosms and mesh bags, we manually separated model particles from the soil for subsequent analysis. (**C**) Self-manufactured model substrate particles. (**D**) Laboratory mesocosm filled with soil and model particles. (**E**) Mesh bag used for the field incubation.

Fig. 2 Bacterial, archaeal, and fungal biomass increased on model particles over the incubation period, but not in the surrounding soil. Boxplots show (**A, C**) 16S rRNA (16S) and (**B, D**) ITS gene copy numbers (per gram of particles or soil) (**A, B**) on model particles and (**C, D**) in the soil after zero, one, 13, 29, and 50 days of lab incubation, and 47 days of field incubation. Gene copy numbers can be interpreted as a proxy for microbial biomass associated with model particles or soil. (**A, B, C, D**) Letters above boxes denote significant differences across timepoints for each treatment and sample type, letters are based on Bonferroni-adjusted p-values of pairwise comparisons that were calculated by performing Dunn´s tests as post hoc test of Kruskal Wallis tests. (**E**) Fluorescence microscopy pictures show microbial DNA at model chitin particle surfaces after one, 13, 29, and 50 days of lab incubation. Yellow points represent DNA stained with SYBR Green I double stranded nucleic acid stain. Microscopy was performed using a Leica confocal laser scanning microscope coupled with a Thunder imaging system. Scale bars indicate 1 µm.

Fig. 3 Bacterial and archaeal, and fungal richness of model chitin particles significantly decreased over the incubation period. Boxplots (from left to right) show (**A**) bacterial and archaeal richness (number of ASVs), and (**B**) fungal richness that was detected in (i) the soil, which was used to set up incubations, (ii) on model particles, and (iii) in the surrounding soil after one, 13, 29, and 50 days of lab incubation and 47 days field incubation. Sequencing data was randomly rarefied at the minimum read count for bacteria and archaea (3015 reads), and for fungi (5676 reads) to guarantee equal sequencing depth of samples. Letters above boxes denote significant differences across timepoints for each treatment and sample type, letters are based on Bonferroni-adjusted p-values of pairwise comparisons that were calculated by performing Dunn´s tests as post hoc test of Kruskal Wallis tests. S –

sieved soil that was used to set up incubations, CH – chitin, CE – cellulose, NS – no substrate (control to account for the effect of the scaffold material of model particles, gellan gum), NP – soil without particles.

Fig. 4 Chitin particle-associated bacterial and archaeal communities underwent temporal changes. PCoA plots show potential dissimilarity of (**A-C, G-I**) bacterial and archaeal, and (**D-F, J-L**) fungal communities (i) associated with model chitin, cellulose or "no substrate" particles or (ii) in the surrounding soil across harvest time points (day one to 50 or 47). Plots also include communities of the five sieved soils (at incubation start, 0d) that were used to set up mesocosms and mesh bags. PCoA plots are based on Aitchison distances (Euclidean distance of clr-transformed sequencing data). The colour of data points indicates the harvest time point, the shape of points depicts the type of incubation (lab or field). CH – chitin, CE – cellulose, NS – "no substrate".

Fig. 5 Model chitin and cellulose particles favored certain bacterial genera compared to the soil, in which particles had been incubated. Plots show genera that were significantly enriched in their relative abundance on (**A**) model chitin, (**B**) model cellulose particles, and (**C**) "no substrate" model particles (controls) compared to the surrounding soil at one day, 13, 29, and 50 days after the start of the lab incubation and 47 days after the start of the field incubation. Bars represent the log fold change, a measure of effect size, in the relative abundance of significantly enriched genera (FDR-adjusted P value < 0.05). Differentially abundant genera were identified using the ANCOM-BC package (Lin and Peddada, 2020). Error bars represent the standard error of the log fold change. We controlled for false discovery rates with the Benjamini-Hochberg multiple test correction. *Aquaspirillum** refers to the *[Aquaspirillum] articum group*, *Rhizobiaceae spp.*** refers to the *Allorhizobium-Neorhizobium-Pararhizobium-Rhizobium* clade. Note that we did not detect any genera that were significantly enriched on model "no substrate" particles after 47 days of incubation in the field. See Fig. S1-S4 for positive and negative log fold changes of genera on model particles compared to the surrounding soil.

Fig. 6 Model chitin and cellulose particles favored certain fungal genera compared to the soil, in which particles had been incubated. Plots show genera which were significantly enriched in their relative abundance on (**A**) model chitin, (**B**) model cellulose particles, and (**C**) model "no substrate" particles (controls) compared to the surrounding soil at one day, 13, 29, and 50 days after the start of the lab incubation and 47 days after the start of the field incubation. Bars represent the log fold change, a measure of effect size, in the relative abundance of significantly enriched genera (FDR-adjusted P value < 0.05). Differentially abundant genera were identified using the ANCOM-BC package (Lin and Peddada, 2020). Error bars represent the standard error of the log fold change. We

controlled for false discovery rates with the Benjamini-Hochberg multiple test correction. Note that we did not detect any genera which were significantly enriched on model “no substrate” particles after 13 and 50 days of incubation in the lab, and 47 days of incubation in the field. See Fig. S5-S7 for positive and negative log fold changes of genera on model particles compared to the surrounding soil.

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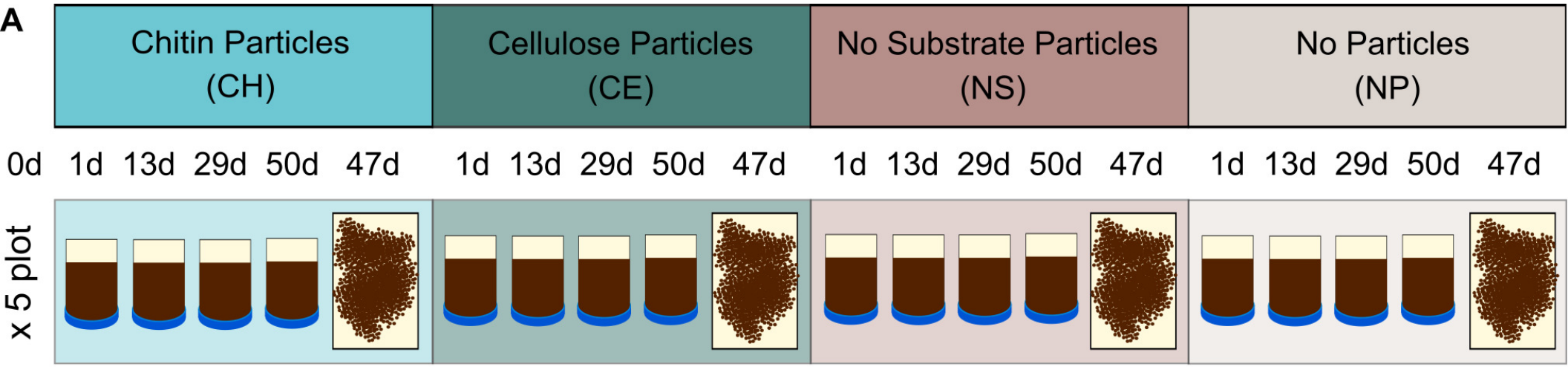
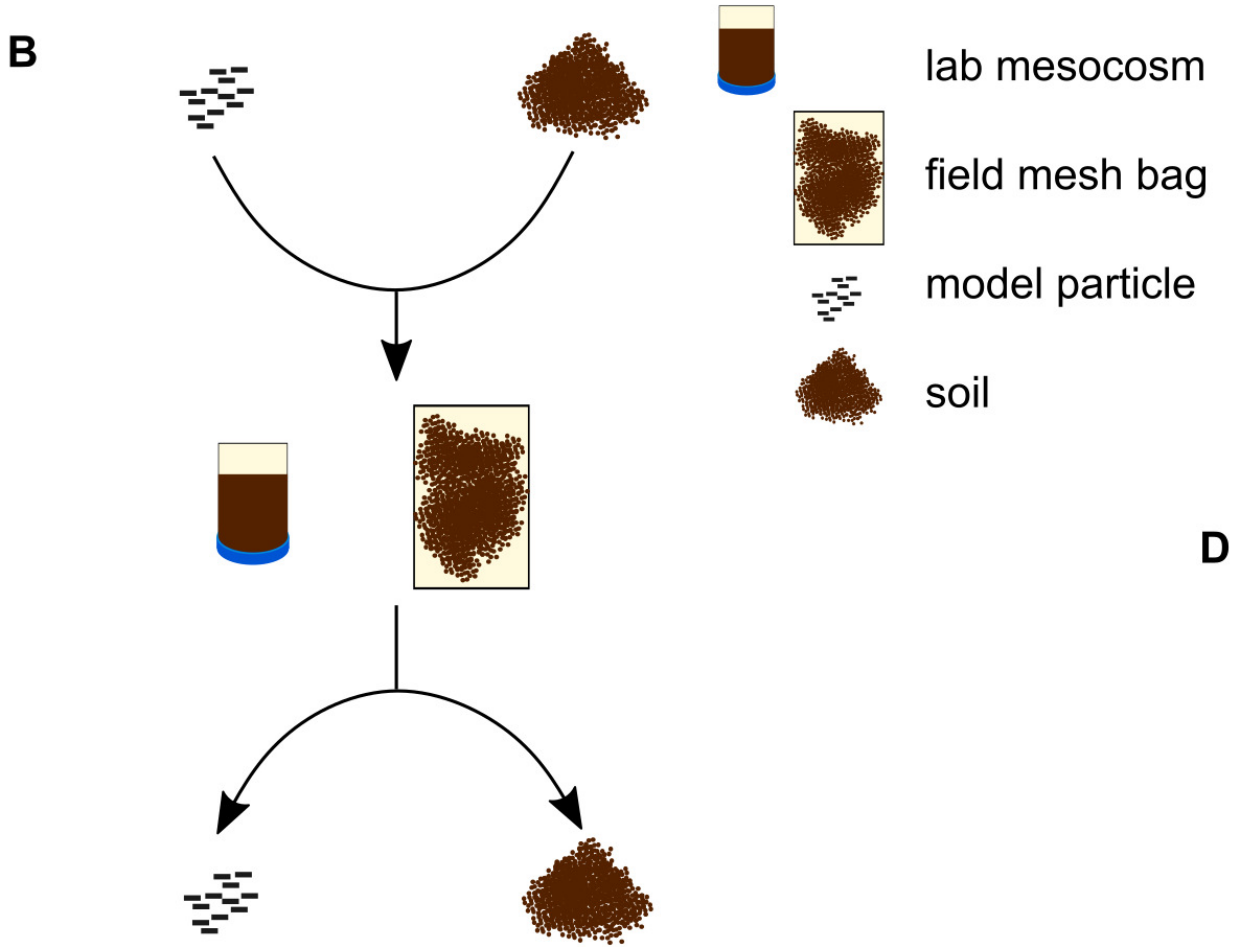
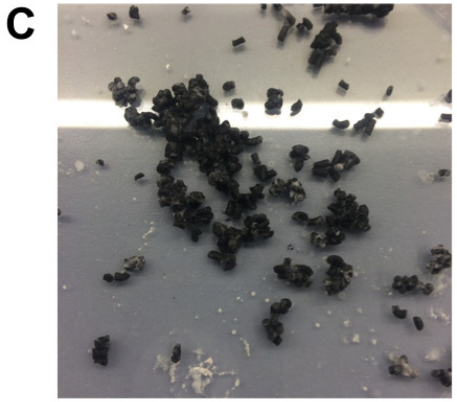
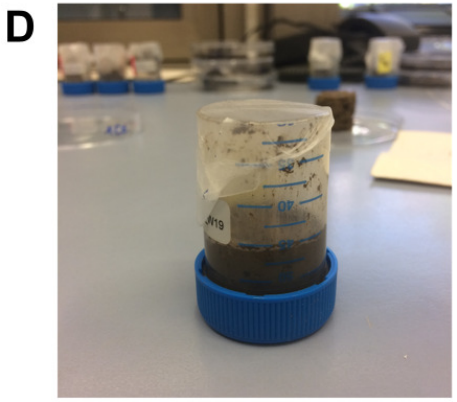
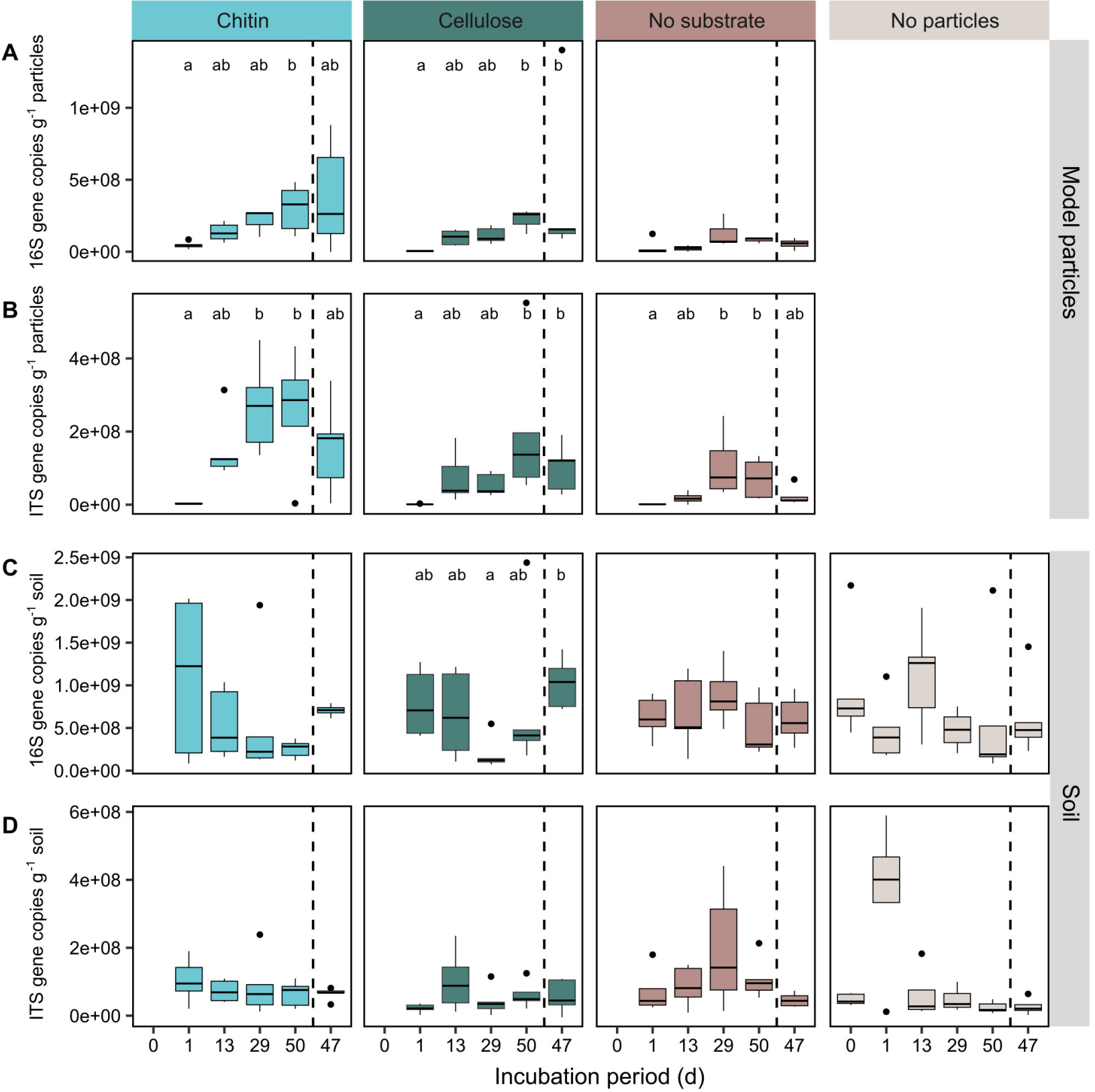

A
Chitin Particles (CH)
Cellulose Particles (CE)
No Substrate Particles (NS)
No Particles (NP)
0d 1d 13d 29d 50d 47d
1d 13d 29d 50d 47d
1d 13d 29d 50d 47d
1d 13d 29d 50d 47d
x 5 plot

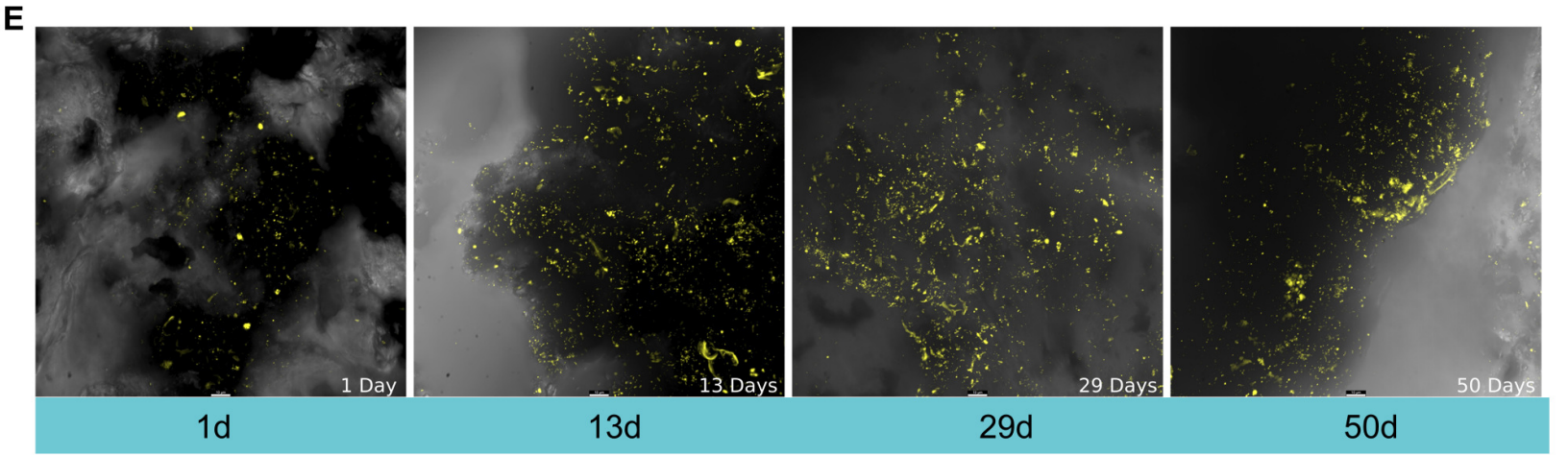

B
lab mesocosm
field mesh bag
model particle
soil

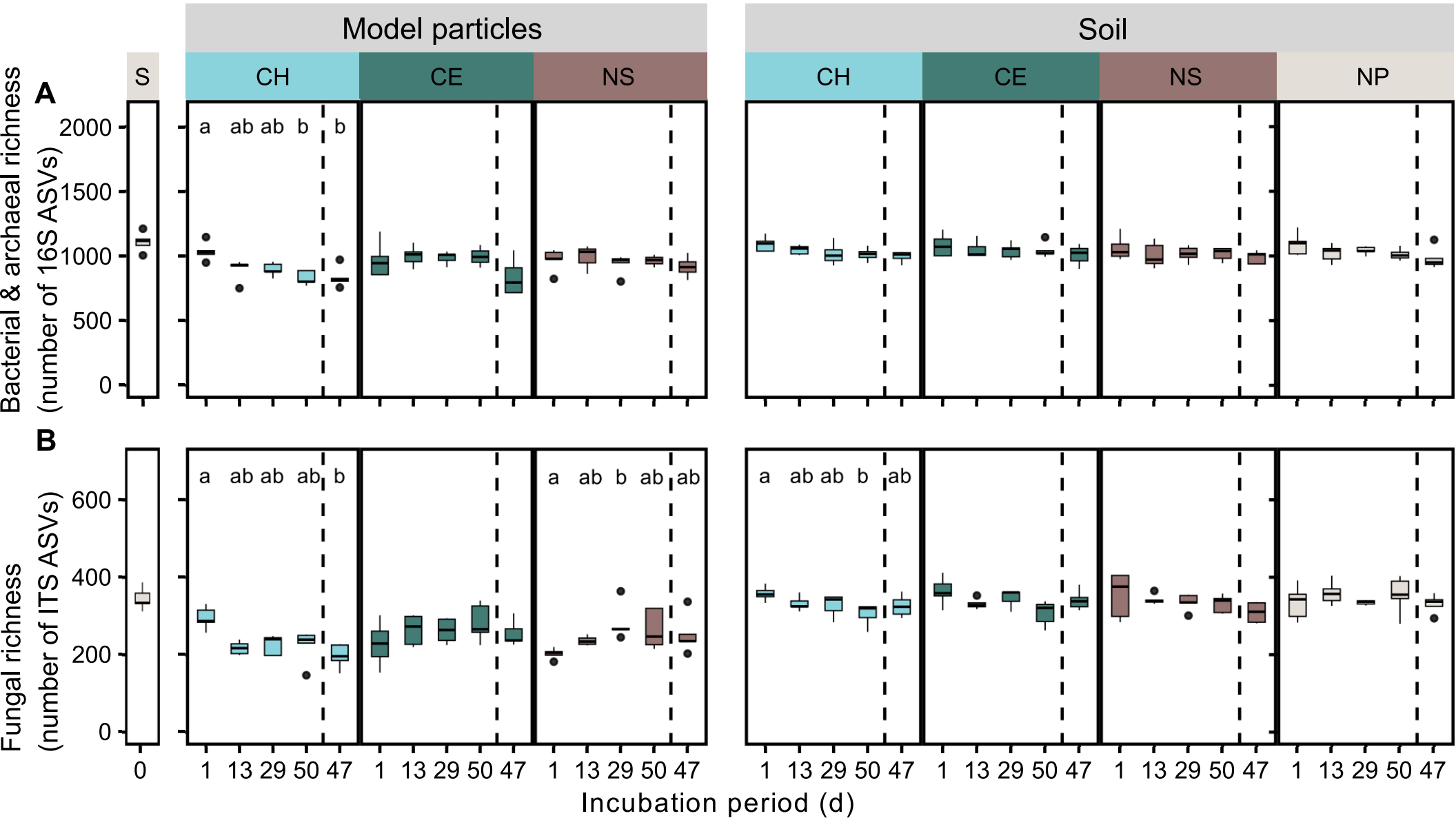

C

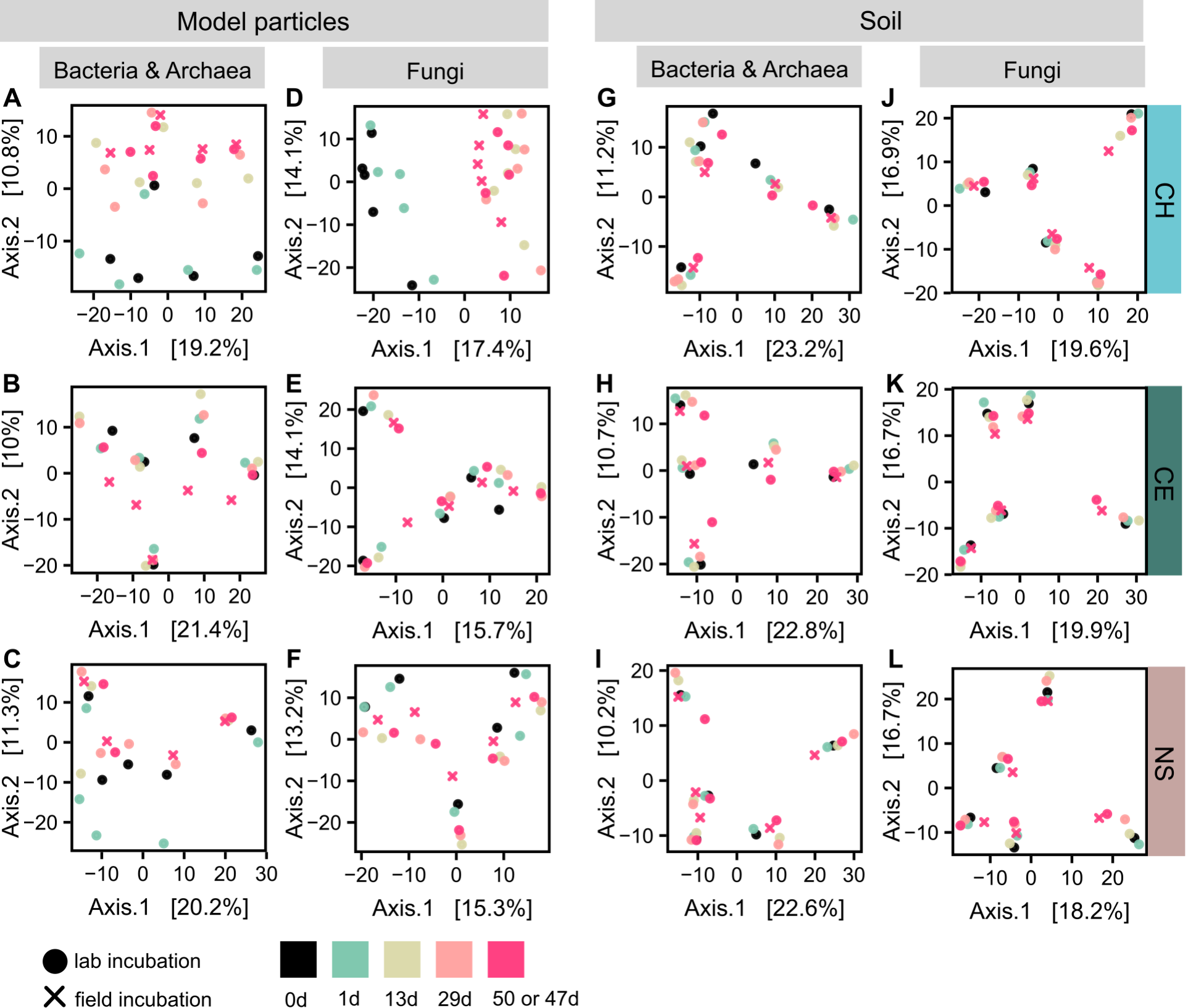

D

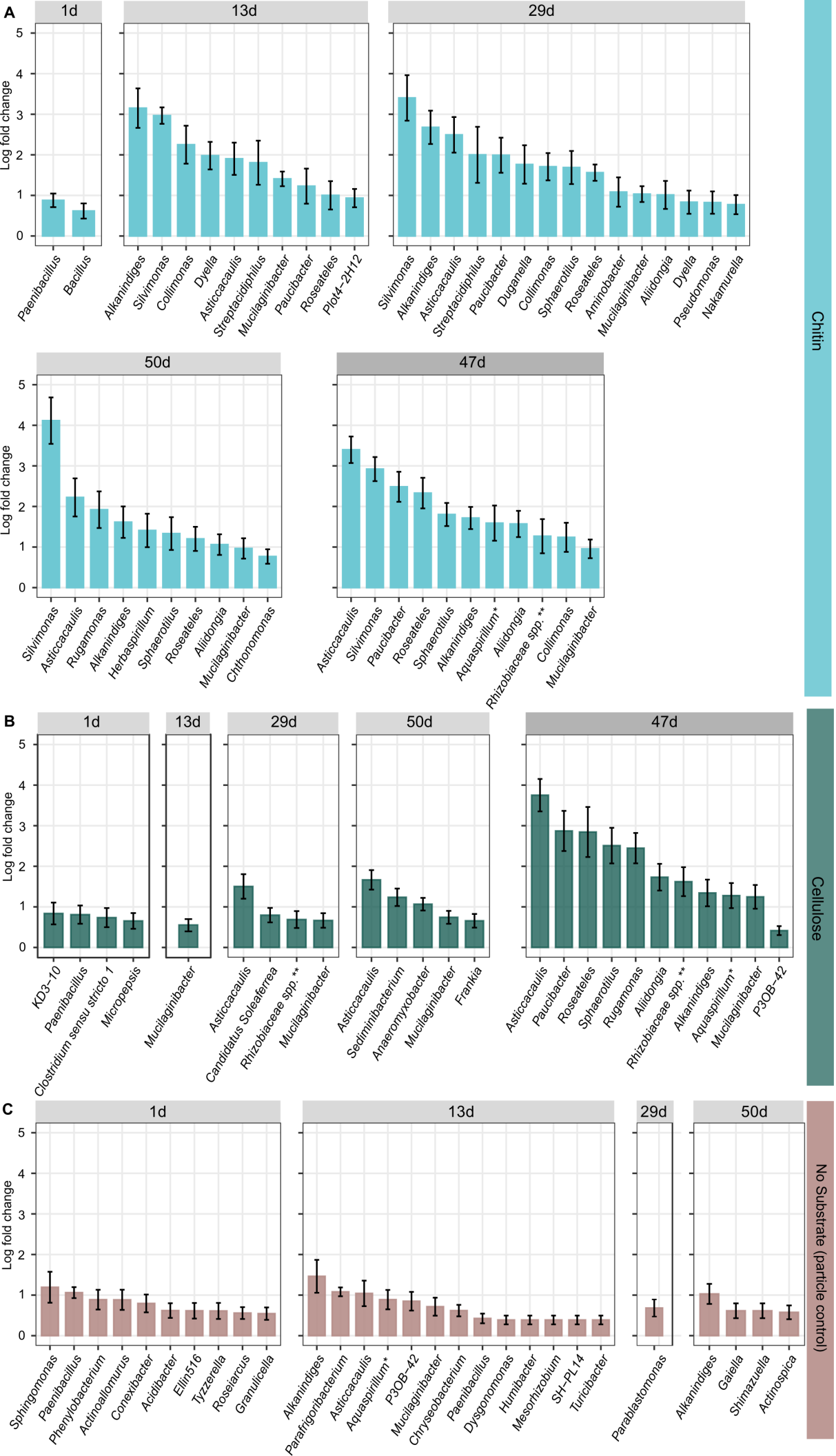
E

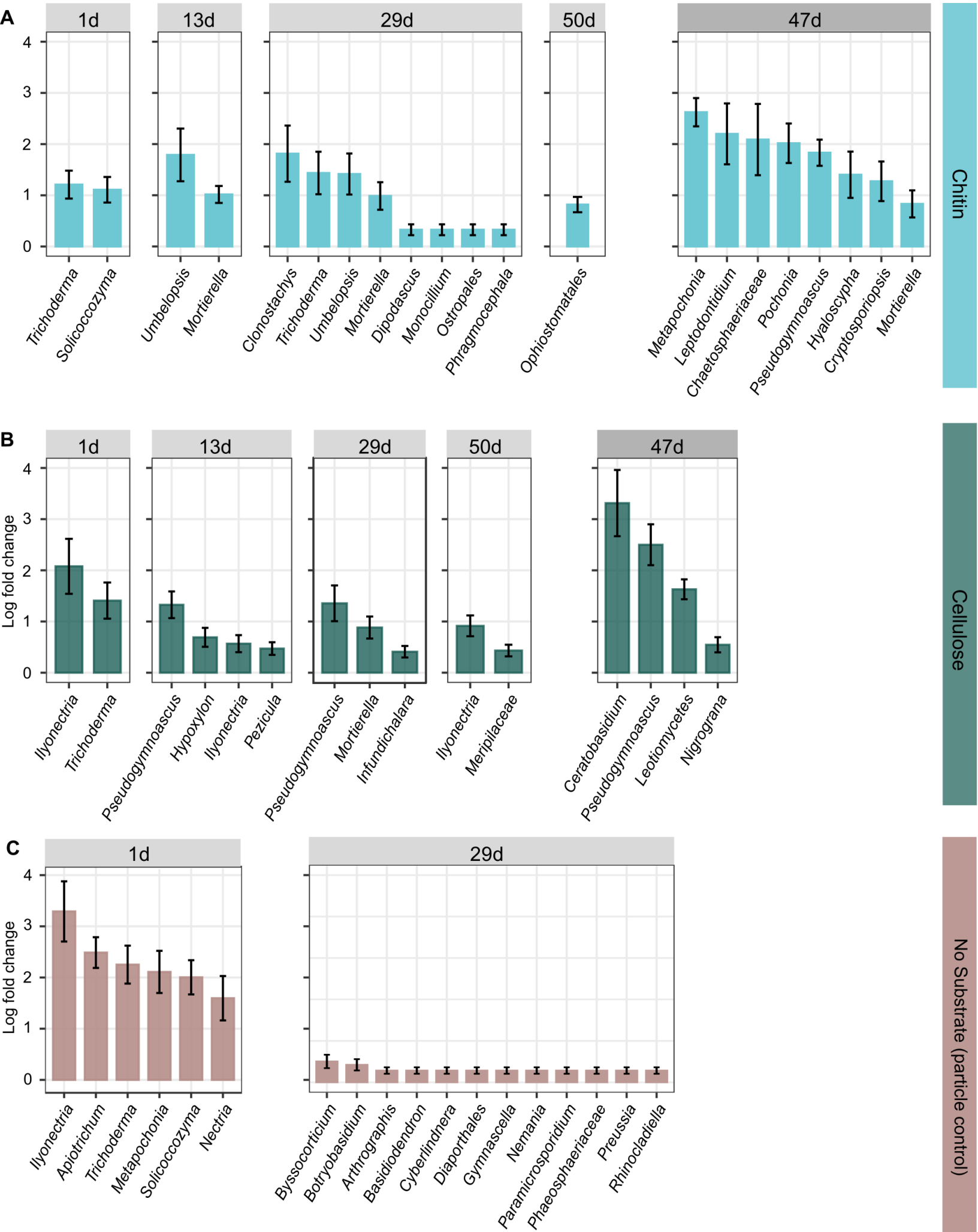

Chitin
Cellulose
No substrate
No particles
A
B
C
D
16S gene copies g-1 particles
ITS gene copies g-1 particles
16S gene copies g-1 soil
ITS gene copies g-1 soil
Model particles
Soil
Incubation period (d)
0 1 13 29 50 47


E
1 Day
13 Days
29 Days
50 Days
1d
13d
29d
50d


Model particles
Soil
S
CH
CE
NS
CH
CE
NS
NP
A
B
Bacterial & archaeal richness
(number of 16S ASVs)
Fungal richness
(number of ITS ASVs)
2000
1500
1000
500
0
600
400
200
0
a ab ab b b
a ab ab ab b
a ab b ab ab
a ab ab b ab
0
1 13 29 50 47
Incubation period (d)


Model particles
Soil
Bacteria & Archaea
Fungi
Bacteria & Archaea
Fungi
A
B
C
D
E
F
G
H
I
J
K
L
CH
CE
NS
Axis.1 [19.2%]
Axis.2 [10.8%]
Axis.1 [17.4%]
Axis.2 [14.1%]
Axis.1 [23.2%]
Axis.2 [11.2%]
Axis.1 [19.6%]
Axis.2 [16.9%]
Axis.1 [21.4%]
Axis.2 [10%]
Axis.1 [15.7%]
Axis.2 [14.1%]
Axis.1 [22.8%]
Axis.2 [10.7%]
Axis.1 [19.9%]
Axis.2 [16.7%]
Axis.1 [20.2%]
Axis.2 [11.3%]
Axis.1 [15.3%]
Axis.2 [13.2%]
Axis.1 [22.6%]
Axis.2 [10.2%]
Axis.1 [18.2%]
Axis.2 [16.7%]
lab incubation
field incubation
0d
1d
13d
29d
50 or 47d


A
1d
13d
29d
50d
47d
Log fold change
Paenibacillus
Bacillus
Alkanindiges
Silvimonas
Collimonas
Dyella
Asticcacaulis
Streptacidiphilus
Mucilaginibacter
Paucibacter
Roseateles
Plot4-2H12
Silvimonas
Alkanindiges
Asticcacaulis
Streptacidiphilus
Paucibacter
Duganella
Collimonas
Sphaerotilus
Roseateles
Aminobacter
Mucilaginibacter
Aliidongia
Dyella
Pseudomonas
Nakamurella
Silvimonas
Asticcacaulis
Rugamonas
Alkanindiges
Herbaspirillum
Sphaerotilus
Roseateles
Aliidongia
Mucilaginibacter
Chthonomonas
Asticcacaulis
Silvimonas
Paucibacter
Roseateles
Sphaerotilus
Alkanindiges
Aquaspirillum*
Aliidongia
Rhizobiaceae spp. **
Collimonas
Mucilaginibacter
Chitin
B
1d
13d
29d
50d
47d
Log fold change
KD3-10
Paenibacillus
Clostridium sensu stricto 1
Micropepsis
Mucilaginibacter
Asticcacaulis
Candidatus Soleaferrea
Rhizobiaceae spp. **
Mucilaginibacter
Asticcacaulis
Sediminibacterium
Anaeromyxobacter
Mucilaginibacter
Frankia
Asticcacaulis
Paucibacter
Roseateles
Sphaerotilus
Rugamonas
Aliidongia
Rhizobiaceae spp. **
Alkanindiges
Aquaspirillum*
Mucilaginibacter
P3OB-42
Cellulose
C
1d
13d
29d
50d
Log fold change
Sphingomonas
Paenibacillus
Phenylobacterium
Actinoallomurus
Conexibacter
Acidibacter
Ellin516
Tyzzerella
Roseiarcus
Granulicella
Alkanindiges
Parafrigoribacterium
Asticcacaulis
Aquaspirillum*
P3OB-42
Mucilaginibacter
Chryseobacterium
Paenibacillus
Dysgonomonas
Humibacter
Mesorhizobium
SH-PL14
Turicibacter
Parablastomonas
Alkanindiges
Gaiella
Shimazuella
Actinospica
No Substrate (particle control)


A
1d
13d
29d
50d
47d
Chitin
Trichoderma
Solicoccozyma
Umbelopsis
Mortierella
Clonostachys
Trichoderma
Umbelopsis
Mortierella
Dipodascus
Monocillium
Ostropales
Phragmocephala
Ophiostomatales
Metapochonia
Leptodontidium
Chaetosphaeriaceae
Pochonia
Pseudogymnoascus
Hyaloscypha
Cryptosporiopsis
Mortierella
B
1d
13d
29d
50d
47d
Cellulose
Log fold change
Ilyonectria
Trichoderma
Pseudogymnoascus
Hypoxylon
Ilyonectria
Pezicula
Pseudogymnoascus
Mortierella
Infundichalara
Ilyonectria
Meripilaceae
Ceratobasidium
Pseudogymnoascus
Leotiomycetes
Nigrograna
C
1d
29d
No Substrate (particle control)
Log fold change
Ilyonectria
Apiotrichum
Trichoderma
Metapochonia
Solicoccozyma
Nectria
Byssocorticium
Botryobasidium
Arthrographis
Basidiodendron
Cyberlindnera
Diaporthales
Gymnascella
Nemania
Paramicrosporidium
Phaeosphaeriaceae
Preussia
Rhinocladiella