Soil microbiomes mediate degradation of vinyl ester-based polymer composites

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Polymer composites are attractive for structural applications in the built environment due to their lightweight and high strength properties but suffer from degradation due to environmental factors. While abiotic factors like temperature, moisture, and ultraviolet light are well studied, little is known about the impacts of naturally occurring microbial communities on their structural integrity. Here we apply complementary time-series multi-omics of biofilms growing on polymer composites and materials characterization to elucidate the processes driving their degradation. We measured a reduction in mechanical properties due to biologically driven molecular chain breakage of esters and reconstructed 121 microbial genomes to describe microbial diversity and pathways associated with polymer composite degradation. The polymer composite microbiome is dominated by four bacterial groups including the Candidate Phyla Radiation that possess pathways for breakdown of acrylate, esters, and bisphenol, abundant in composites. We provide a foundation for understanding interactions of next-generation structural materials with their natural environment that can predict their durability and drive future designs.
Fiber-reinforced polymer composite materials (referred to as polymer composites) have become an attractive option for infrastructure due to their lightweight, high stiffness, and high strength to weight ratios, which are favorable properties for enabling high fuel efficiency and toward increasing mobility. In addition, polymer composites have extremely high corrosion resistance compared to conventional materials like steel which are easily corroded. Therefore, although initial installed costs of polymer composites and steel are comparable, life-cycle costs of polymer composites are expected to be significantly lower. Currently, polymer composites have a prevalent use in a large range of infrastructure including pipelines, utility poles, prefabricated pavements, renewable energy harvesting, chimneys or flues, rapidly deployable housing, decking for marine and naval structures, and advanced retrofitting. Recent studies have demonstrated that polymer composites, when designed appropriately, can provide a cost-effective alternative to current structural materials used for primary loading-bearing elements in civil and space infrastructure. Specific civil infrastructural applications of polymer composites include but are not limited to retrofitting of existing concrete structures, strengthening of steel structures, as internal reinforcing rods in reinforced concrete structures, in new construction as structural members in supporting frame structures, multi-storey office and residential buildings, bridge decks for pedestrian and highway bridge superstructures, electricity transmission towers, and composite pile caps for foundation construction. Given their prevalent use in current and future human establishments, it is of utmost importance to understand and elucidate their long-term durability and survivability in natural environments.

Polymer composites are composed of several individual constituent materials with unique properties that are combined together to achieve improved physical properties as compared to the individual materials. They typically consist of two distinct components, a matrix and reinforcing materials. Reinforcing materials such as fibers provide strength to the composite, while the matrix acts as a binding agent to hold the composite together. A common type of polymer composites are fiber-reinforced polymer composites where fibers such as those made of carbon act as reinforcements in a polymer matrix, and polymers such as epoxy, vinyl ester, and polyesters are used as matrix. Carbon fibers are widely used for reinforcement due to their high-strength and lightweight properties, and vinyl ester is widely used as binding matrices due to better resistance to moisture absorption and ultraviolet (UV) radiation compared to polyesters and other binding agents. Nevertheless, polymer composites are susceptible to degradation by the chemical, physical, and biological stressors in the environment. Key environmental factors that can influence the durability of polymer composites include moisture, temperature, pH, salinity, sustained stresses, and microbes. The response of polymer composites to a few of these environmental factors, like temperature, moisture, pH, freeze–thaw cycles, acting independently or in combination have been well studied. However, the influence of microbial interactions on the durability and longevity of polymer composites has seldom been studied. In this current study, we investigate the durability and survivability of a commonly used polymer composite with woven carbon fibers as the reinforcement in vinyl ester polymer matrix.

Previous studies have demonstrated that incubation of binding matrices such as epoxy with individual organisms such as Pseudomonas spp. as well as simple consortia of microorganisms can degrade these compounds. These microorganisms typically formed a viscoelastic layer or biofilm on the material surface. Possible mechanisms for microbial degradation of materials involved the degradation of organic polymers in the binding matrix involving direct attack by acids or enzymes, blistering due to gas evolution, enhanced cracking due to calcareous deposits and gas evolution and polymer destabilization by concentrated chlorides and sulfides.

Among these very limited studies on polymer composites, most have focused only on the influence of single microorganisms on specific compounds such as epoxy and vinyl ester in controlled environments. Wagner et al. exposed vinyl ester polymer composites to four different microbial cultures, namely Thiobacillus ferroxidans (a sulfur/iron oxidizing bacterium), Pseudomonas flourens (a calcareous-depositing bacterium), Lactococcus lactis (ammonium and sulfide producing bacterium), and sulfate-reducing bacteria (SRB; producing sulfide from sulfate), on polymer composites. Bacterial colonization of fibers and composites were observed. Hydrogen (H2)-producing bacteria appeared to disrupt bonding between fibers and vinyl ester resin in the polymer composites and penetrated the resin at the interface in addition to disrupting fibers and resin due to gas formation within the composite. The strength of polymer composites reduced after exposure to hydrogen sulfide (H2S) (from Lactococcc and SRB) and its corrosive effects. In addition, polymer composites can also be impacted by leaching activities of heterotrophic bacteria that seek carbon from the polymer and production of reactive oxygen species during growth. Carbon is a critical nutrient necessary for microbial growth and organic components in binding matrices such as epoxy can serve as the sole source of carbon for organisms found in soil such as Rhodococcus rhodochrous and Ochrobactrum anthropicum.

In addition to bacteria, a few studies have also investigated the impact of fungi on polymer composites. Investigations using a fungal consortium (Aspergillus versicolor, Cladosporium cladosporioides, and Chaetomium) on different polymer composites revealed that all samples were colonized by the consortia with fungal penetration along the fibers accelerating the attack on the binding matrix. It is likely that organic compounds in the polymer composites likely served as carbon and energy sources for the growth of fungi. The versatility of polymer composites has enabled their use in dental resins. Recent studies have investigated the influence of proteins, in lieu of microorganisms on polymer composites. Cai et al. studied the influence of human saliva on degrading ester linkages in dental resin monomers. The study reported that the formation of proteins such as albumin and the Zn-a2-glycoprotein from human saliva could degrade the ester linkages by enhancing the ester cleavage reaction that eliminated the esters from the reactive site of albumin’s esterase activity. Several recent studies reported that vinyl ester polymer composites are susceptible to structural degradation due to UV irradiation, soil burial, alkaline solution submersion, salt exposure, and regular water immersion, which causes hydrolysis of the vinyl ester resin and subsequently reduces the mechanical and thermal performance of the overall composite structures.

Though important, these single organism and protein-based studies do not adequately capture the impacts of a complex natural microbial community on polymer composites. Moreover, much of these organisms used in prior studies are not abundant in molecular surveys conducted in nature (for example in soils) and are thus unlikely to be the key drivers of degradation of composites. Therefore, little is known about the diversity and impacts of microbes interacting with polymer composites in nature. Cultivation-independent studies that can comprehensively characterize the microbial community and its deleterious effects on composites are needed to understand their impacts and design mitigation strategies.

Here, we study the impact of a complex natural microbial community on the degradation of vinyl ester carbon fiber
polymer composites over time. We performed complementary mechanical and materials characterization and multi-omics analyses to assess the extent and type of degradation of the polymer composites and obtain a genome-informed perspective on the potential role of microorganisms in this process. By utilizing a time-course experiment, we were able to observe a gradual reduction in a number of metrics associated with strength, stiffness, durability, and survivability of polymer composites. Our sophisticated materials characterization approach revealed for the first time, specific mechanisms that underpinned degradation of polymer composites by microorganisms over time. Our microbial community composition and metagenomic analyses support our findings from mechanical and materials characterizations. We observed the presence of a stable biofilm on the polymer composites and utilized genome-resolved analyses to demonstrate the putative roles of poorly studied microbes, including a significant proportion from uncultivated candidate phyla in the degradation of polymer composites. Overall, our study demonstrates that uncultivated microbes in nature possess the ability to degrade widely used polymer composite materials in the built environment.

Results
Exposure study of polymer composites: sampling the polymer composite microbiome and materials characterization. We used microbial community analyses based on 16S ribosomal RNA sequencing and genome-resolved metagenomics, and mechanical and materials characterization based on thermogravimetric analysis (TGA) and Fourier-transform infrared spectroscopy (FT-IR) to study the impacts of microorganisms on the degradation of polymer composite materials. We fabricated vinyl ester-based carbon fiber-reinforced composites in-house using vacuum-assisted resin transfer molding process, which is commonly used for fabricating such composites. These composites were fabricated using layers of woven carbon fiber bundles and stacking them in the thickness direction, followed by infiltration of vinyl ester resin through the dry fabric. Solid composites were obtained upon curing of the resin with the carbon fiber layers.

Optical and scanning electron microscopy (SEM) images of the fabricated polymer composite are shown in Fig. 1 at different length scales. We inoculated these polymer composites with a soil solution (Soil with deionized water, S+DI) containing soil samples collected from an area adjacent to Lake Mendota, WI, USA (Fig. 2), for 24 weeks. The samples were inoculated in sealed bottles in the presence of light that were aerobic initially but allowed to go anaerobic over time. We hypothesized that these soil samples contained a diverse microbial community that would colonize the polymer composites. Two types of control samples were maintained and analyzed over the course of the experiment. First, polymer composites were incubated in autoclaved soil solution with deionized water (S+DI+A). These samples allowed us to measure the impact of soil chemistry (without microorganisms) on the polymer composites. Secondly, polymer composites were incubated in autoclaved DI water (DI+A) to measure the impacts of water on polymer composites (without microbial and chemical impact). Immediately post-inoculation, we observed the presence of a biofilm on the polymer composites incubated in soil with deionized water. No biofilms were observed in the two control samples. The experimental and control groups were observed over a time course of 24 weeks and samples for microbial and materials characterization were collected every 2 weeks.

Three different materials characterization procedures, including TGA, FT-IR, and Nanomechanical Characterization, were performed on polymer composites. Possible leaching of carbon from the composites into the solution was measured using dissolved organic carbon (DOC) analysis on liquid samples from the incubation solution toward the end of the tests. From these characterizations, we were able to clearly show that the manifestation of polymer composite degradation primarily resulted from microbial activity.

For microbial characterization, we performed 16S ribosomal RNA amplicon sequencing to determine the structure and abundance of the microbial community, and metagenomic sequencing to profile the functional capacity of microorganisms to degrade polymer composites. To the best of our knowledge, no prior studies have investigated the combined effect of a diverse
Degradation of polymer composites in the presence of microbes. We performed TGA upon exposure of polymer composites to different environments mentioned above to establish the extent of degradation over varying exposure periods and to shed light on the polymer degradation mechanisms. The extent of degradation in polymer composites was measured in terms of thermal decomposition onset temperature, which is expected to reduce with higher degrees of degradation. Typical output from a TGA is the weight loss percentage of a material when heated to high temperatures, typically in the range of 100–400 °C which are plotted in a graph. The temperature at which the slope of this graph changes significantly (knee formation highlighted in Fig. 3a) is referred to as the thermal decomposition onset temperature. We collected samples from regions near the surface of polymer composite samples to conduct TGA. Figure 3a (Supplementary Data 3 Table 1) compares the TGA graphs of samples in soil solution (S+DI) periodically from week 10 to week 20. We observed that the thermal decomposition onset temperature reduced with increasing exposure time. This was attributed to the hydrolysis of certain groups (e.g. ester groups, C=O groups) in the polymer chain that weakens their backbone structure, resulting in polymer chain scission generating/forming low molecular-mass compounds. Next, we compared the thermal decomposition onset temperatures for samples from week 10 to week 20 against exposure time for all environments in Fig. 3b (Supplementary Data 3 Table 2). We observed that this onset temperature reduced significantly for polymer composite samples in soil solution (S+DI) as compared to autoclaved water (DI+A) and autoclaved soil with water (S+DI+A) conditions. We observed that the presence of microorganisms (in the [S+DI] samples) strongly correlated with a higher degree of degradation of polymer composites as compared to other conditions where no or minimal microbial growth and activity was possible. Hence, we conclude that microbial activity drives the polymer chain scission-based degradation of polymer composites, which was manifested as a substantial reduction of the onset melting temperature. We further verified these findings by determining the average molecular weight of the composite samples exposed to soil solution (S+DI), which were approximately 2793, 2364, and 2138 g mol⁻¹, respectively, for 0, 10, and 20 weeks samples as shown in Fig. 3c. It is evident that the average molecular weight of the polymer composite significantly decreased with time. This lends further support to our claim that microorganism-mediated chain scission activity is responsible for the pronounced polymer composite degradation process.

To establish the adverse influence of microbial degradation on the mechanical properties of polymer composites, we performed surface nanoindentation tests on exposed vinyl ester polymer composite samples. Reduction in mechanical properties due to degradation is measured in terms of modulus, hardness, and displacement upon nanoindentation of polymer composites, where the reduction in modulus and hardness, and increase in displacement are manifestations of polymer degradation. A comparison of load against displacement is a typical output from nanoindentation tests. Representative load–displacement graphs from nano-indentation tests on vinyl ester polymer composite samples exposed to soil solution (S+DI) from weeks 0, 8, 16, and 24 are shown in Fig. 4a (Supplementary Data 3 Table 3). Other representative load–displacement graphs from nano-indentation tests on vinyl ester polymer composite samples exposed to soil...
with autoclaved (S+DI+A) and deionized autoclaved (DI+A) solutions are shown in Supplementary Fig. 1. Modulus, hardness, and displacement against the number of weeks were extracted from such graphs shown in Fig. 4a and Supplementary Fig. 1, which were then plotted in Fig. 4b–d. We observed that the modulus and hardness decreased drastically with increasing number of weeks of exposure to soil solution compared with the soil with autoclaved (S+DI+A) and deionized autoclaved (DI+A) solution, while the displacement increased. These results demonstrate that the surface nanomechanical properties of vinyl ester polymer composites were adversely affected by the degradation caused by microbial activity.

Microbial diversity and abundance in the polymer composite microbiome. Given the presence of significant damage to polymer composites in the presence of soil, we hypothesized the involvement of microbial communities in their degradation. To determine the stability and diversity of the microbial community inhabiting the polymer composite biofilm, we collected biofilm samples every 2 weeks and used 16S rRNA amplicon sequencing to determine community composition. In total, we generated 2.2 million paired-end reads. Processing these reads revealed the presence of 8461 operational taxonomic units (OTUs) which is an estimate of the number of microbial genotypes in the polymer composite microbiome (Supplementary Data 3 Table 4). Samples from weeks 0, 2, and 4 were distinct from other samples suggesting the recruitment of microbes into the biofilm from the soil samples used for inoculation (Supplementary Data 3 Tables 5 and 6). Microbial community composition and relative abundance across all sampling weeks are displayed in Fig. 5. From weeks 6 to week 24, the microbial community inhabiting the polymer composite biofilm was distinct from the first three samples. In total, we found 446 OTUs to be unique to weeks 0, 2, and 4 while samples from weeks 6 to 24 had a total of 204 unique OTUs (Supplementary Data 3 Tables 5 and 6). The majority of the OTUs from weeks 6 to 24 remained a stable component of the biofilm, but their abundance varied over the course of the 24 weeks. In total, only 8% of OTUs from week 0 were observed in week 24. Microorganisms from the bacterial lineages Chlorobi, Deltaproteobacteria, Candidate Phyla Radiation/Patescibacteria, and Chloroflexi were the most abundant members of the biofilm.

To infer the metabolic capabilities of the dominant members of the biofilm to impact the polymer composite, we conducted metagenome sequencing on a subset of samples chosen from our microbial community composition analyses. We chose two samples from weeks 8 and 18 that represent the average microbial community across the 24 weeks of our experiment. Metagenome sequencing resulted in ~85.9 Gbp of paired-end reads. After read processing and assembly, we generated ~100,000 assembled DNA sequences (scaffolds) representing 1.74 Gbp of sequence (>1 Kb in length). To generate microbial genomes from assembled DNA sequences, we utilized a comprehensive genome binning and consolidation approach. This resulted in reconstruction of 144 metagenome-assembled genomes (MAGs), in total of which 51 MAGs were classified as high-quality and 70 MAGs as...
medium-quality drafts in accordance with MIMAG standards. These 121 MAGs were used for downstream analyses of elucidation of metabolic potential and polymer composite degradation mechanisms of the biofilm. To undertake taxonomic classification of these genomes, we used two distinct phylogenetic reconstruction approaches including 16 concatenated ribosomal proteins and the Genome Taxonomy Database toolkit (GTDB-tk). Consistent with results from our 16S rRNA amplicon study, our phylogenetic analyses revealed that most MAGs represented the bacterial lineages Chloroflexi (17 MAGs), Chlorobi (33 MAGs), Deltaproteobacteria (24 MAGs), and Candidate Phyla Radiation (CPR)/Patescibacteria (25 MAGs) as shown in Fig. 6a. Within the highly diverse CPR/Patescibacteria group, we recovered MAGs from the lineages Gracilibacteria, Moranbacteria, Saccharibacteria, Roizmanbacteria, CPR1, WWE3, Falkowbacteria, and Yonathbacteria (Supplementary Data 3 Table 7).

To estimate the relative abundance of all organisms in the biofilm, we conducted read-mapping to estimate the coverage of individual MAGs across weeks 8 and 18. Our results show that MAGs from four bacterial lineages Chloroflexi, Chlorobi, Deltaproteobacteria, and CPR/Patescibacteria accounted for 91–95% of the total microbial abundance (Fig. 6b, c and Supplementary Data 3 Table 8). Specifically, organisms from the CPR/Patescibacteria group comprised 30–43% of the abundance. Nearly, the entire abundance of CPR/Patescibacteria could be attributed to organisms from the lineages, Candidatus Gracilibacteria, Candidatus Moranbacteria, Candidatus Yonathbacteria, and Candidatus Saccharibacteria.

Metabolic potential of the polymer composite microbiome. The 121 high- and medium-quality MAGs from the polymer composite microbiome encoded a total of 275,719 protein-coding open reading frames. To determine important metabolisms encoded by the abundant organisms in the biofilm, we screened these proteins for their association with metabolic pathways. This approach served two key purposes. First, the association of dominant organisms with metabolic pathways sheds light on potential biological processes that occur in polymer composite biofilms in nature. Second, this allows us to identify specific metabolic pathways that are associated with the breakdown of individual components in polymer composites. Importantly, we identified energy metabolism and key ecosystem functions being conducted by organisms in the biofilm that can shed light on their
roles, and their importance to degradation of polymer composites. Metabolism encoded in the polymer composite micro-biome included sulfate reduction, sulfur oxidation, fermentation, nitrogen fixation, carbon fixation, and organic carbon oxidation (Supplementary Data 3 Tables 9–12). The metabolic potential for sulfate/sulfite reduction was observed in organisms from Delta-proteobacteria, Nitrospirae, and Firmicutes. Sulfur oxidation was observed in organisms from eight different phyla including Chlorobi and Betaproteobacteria. In accordance with previous observations, most Chlorobi organisms were phototrophs also conducting sulfide oxidation. Fermentation, primarily acetogenesis was observed in organisms from 18 different phyla including CPR. While other organisms from other phyla complemented their metabolism with fermentation, CPR organisms were not observed to possess any alternate forms of metabolism (Supplementary Data 3 Tables 9 and 10). The ability to fix nitrogen was observed in 58 MAGs from ten distinct lineages. Finally, carbon fixation was a commonly observed trait in the microbial community. Our results show that the composite biofilm was not dominated by a single metabolism but was instead highly connected and diverse metabolisms associated with carbon, nitrogen, and sulfur transformations contributed to energy metabolism.

Structural chemical composition changes in polymer composites due to microbial activity. To understand the structural changes in polymer composites induced by microbial activity, we performed a detailed surface chemical analysis using FT-IR (Supplementary Data 3 Tables 13–15). A typical output of FT-IR is a comparison of absorbance intensity with respect to wavenumber (frequency) (Fig. 7a and Supplementary Figs. 4, 5), which is a result of the interaction of infrared radiation between the chemical bonds at different frequencies. The structural chemical changes before and after exposure to different conditions were analyzed. Figure 7a shows superimposed FT-IR spectra of a polymer composite sample exposed to soil solution for different exposure times. The spectra show several peaks corresponding to different wavenumbers that manifest due to the interaction of infrared radiation between chemical bonds at different frequencies. Changes in chemical bonds are reflected as changes in peak intensity, broadening, and shifting with respect to the peaks expected at specific frequencies corresponding to a particular type of chemical bond. Peak broadening and higher peak intensities are associated with increased formation of intramolecular or inter-molecular hydrogen bonding.

In Fig. 7b, c (Supplementary Data 3 Tables 16 and 17), C=O stretching and –OH stretching absorbance intensities were summarized for samples exposed to different conditions over the period of 20 weeks. The absorbance intensities increased with exposure time when compared to samples from week 0, indicating a change in the chemical molecular structure. In particular, this change was significantly higher for samples in the presence of microbial activity as compared to where microorganisms were absent, or in controls.

To further elucidate the impact of microbial action on the degradation of polymer composites, we analyzed the DOC content in each of the solutions after conditioning the composite samples. In general, the DOC content in the solution should increase based on the chain scission mechanism associated with hydrolysis of esters. Here, we performed DOC analysis to support and confirm the chain scission mechanism described in Fig. 8a. Water molecules preferentially establish a weak hydrogen bond with the vinyl ester polymer, which manifested as peak broadening of the –OH stretching from FT-IR analyses. Weak hydrogen bonding promotes the chain scission process over time that decreases the average molecular weight of the polymer composites as shown in Fig. 3c. This eventually leads to reduced surface nanomechanical properties such as lower hardness and modulus, which was confirmed by nanomechanical characterizations in Fig. 4.

As seen in Fig. 8b (Supplementary Data 3 Table 18), the DOC content increased with increasing exposure time for composite samples under different conditions. However, the increase in
DOC in soil solution with time is not as pronounced as compared to other conditions. This is attributed to the presence of microbes that consume/alter the DOC content over time, and a cyclic recovery of DOC is needed to maintain equilibrium in the solution. This phenomenon results in a cyclic process of further chain scission, DOC consumption/alteration, and microbial growth, which causes severe degradation of the polymer composites. In other cases, without microbes, DOC content increases significantly as there are no microbes present to consume or alter the DOC content. In these cases, the chain scission process will stop in order to maintain equilibrium, and the mechanism proposed in Fig. 8a will not continue to degrade the polymer composite.

Potential contributions of microbial metabolism to degradation of polymer composites. To understand the microbial mechanisms driving the degradation of polymer composites, we identified metabolic pathways in microbial genomes that can transform or breakdown individual components of polymer composites. In total, we identified six unique metabolisms that potentially had detrimental effects on the structure of the polymer composite by causing breakup of the physical structure through gas formation and blistering, and degradation of organic compounds in the matrix through chain scission and other processes (Table 1 and Supplementary Data 3 Table 19). Prior research has suggested that production of gases such as hydrogen and H₂S gases can degrade the binding matrix and decrease the structural integrity of the composite (Table 1 and Supplementary Data 3 Table 19). The metabolic capability to produce hydrogen was identified in 24 MAGs from 11 different lineages involving three hydrogen-evolving hydrogenases, including two families of [FeFe] hydrogenase and a group 4 [NiFe] hydrogenase (Supplementary Data 3 Table 9). Amongst the four most abundant lineages in the composite microbiome, we detected this capacity in MAGs from CPR/Patescibacteria, Deltaproteobacteria and Chloroflexi. Two distinct microbial
pathways can produce H2S by the anaerobic respiration of sulfate/sulfite, or by degradation of the amino acid cysteine. While both of these metabolic capacities were detected in the composite microbiome, degradation of cysteine was observed to be more abundant. The ability to respire sulfate/sulfite and produce H2S was observed only in MAGs from the lineages Deltaproteobacteria, Nitrospirae, and Firmicutes.

We identified four components of the polymer composite binding matrix as capable of being degraded by microbial action, including methyl acrylate, bisphenol A, methyl ethyl ketone, and esters. Amongst these, the most abundant capacity in the composite microbiome was the ability to degrade acrylate through the activity of an amidase, which was observed in 77% of all MAGs (Supplementary Data 3 Table 20). The ability to degrade bisphenol A and esters were less common in the biofilm and identified in seven MAGs each. No pathways for the degradation of methyl ethyl ketone were detected in any of the organisms. Since we did not directly measure the activity of these processes, we estimated growth rates of microorganisms likely to be associated with degradation of composites. This was performed using a recently demonstrated approach where it was shown that growth rates of microbial strains in their natural environment can be determined by measuring the ratio of the coverage of DNA at the origin and terminus of replication in a genome. We observed that microorganisms from all four abundant lineages, Chlorobi, Chloroflexi, Deltaproteobacteria, and the CPR/Patescibacteria were growing actively. We propose that a significant proportion of these organisms likely source carbon from the polymer composites and their high rates of growth are associated with breakdown of the polymer composite (Supplementary Fig. 6).

Discussion
Polymer composites are being used increasingly in lightweight structural applications in the built environment and will likely be a backbone of next-generation materials. Therefore, significant resources and research are needed to understand the performance and durability of these materials under physical, chemical, and biological stressors that can induce damage and impact their durability. Our goal of this study was to elucidate biologically driven degradation mechanisms of polymer composites. To that end, we studied the degradation of polymer composites in the presence of microbial communities, and we identified key modes of biologically driven degradation mechanisms of polymer composites. To that end, we studied the degradation of polymer composites in the presence of microbial communities, and we identified key modes of biologically driven degradation which are likely associated with selective microbial enzymes.

Our temporally resolved experimental design involved inoculation of polymer composites with microbial communities from natural soils. We recorded the early onset and growth of a biofilm on the surface of these polymer composites. Overall, we observed
a significant reduction in the molecular weight of the vinyl ester resin used as a binder in polymer composites. With increasing exposure time to microbes, a greater reduction in molecular weight was observed. We propose this to be a manifestation of chain scission in the vinyl ester resin of the polymer composites. Vinyl ester resins are composed of a number of organic compounds, of which acrylate, bisphenol, and esters are the most abundant based on known compositions and existing literature. Specifically, we observed that certain bonds such as C=O, aromatic and aliphatic C–H, O–H, and –OH, which are common in the vinyl ester resin were specifically targeted by microbial activity. We identified four microbial groups to be especially abundant components of the microbial biofilm, namely CPR/Patescibacteria, Deltaproteobacteria, Chlorobi, and Chloroflexi and contributed toward acrylate degradation, bisphenol A degradation, breakdown of esters, hydrogen production, and H2S production.

Amongst the microbial metabolisms associated with degradation of the binding matrix, the most abundant capacities in the composite microbiome were associated with degradation of acrylate, and production of H2S (Fig. 9). The ability to degrade bisphenol A and esters, and produce hydrogen were less common in the biofilm. We speculate that microorganisms that can degrade acrylate and/or produce H2S are selected for in this environment. There are three potential factors that support these observations. First, acrylate degradation is primarily mediated by amidases that are abundant in these specific microbial groups. While amidases are primarily associated with degradation of amide containing compounds such as urea for sourcing nitrogen, we propose that the composite microbiome utilizes these for degradation of acrylate in the vinyl ester resin. Second, H2S may be produced by dissimilatory sulfate reduction by organisms from the Deltaproteobacteria and Nitrospira lineages. Prior research has demonstrated gradients of oxygen in biofilms including the presence of anoxic regions that could support highly active sulfate reducing microbes. Third, H2S may also be produced by degradation of cysteine which is likely an abundant component of organic-rich biofilms. We propose that the interplay between the degradation of the binding matrix (acrylate, bisphenol A, esters) through chain scission of chemical bonds and the production of gases (H2S, H2) that can cause physical damage and potential bloating of the polymer structure contributes toward accelerated degradation of polymer composites.

Our study builds on prior research involving studies of single microorganisms such as Pseudomonas, Lactococcus, SRB, Rhodococcus, and Ochrobactrum species that can degrade polymer composites. Identification of microorganisms from four bacterial phyla, namely, Deltaproteobacteria, Chlorobi, Chloroflexi, and CPR/Patescibacteria as dominant components of the microbial biofilm on polymer composites provides vital insights that could direct approaches for testing the durability of these structures in the field. Organisms from these bacterial lineages are abundant in a variety of terrestrial, marine and coastal environments where they could degrade polymer composite-based infrastructure. In remote field applications, such as in pipelines and wind turbines, any observed biofilms on the composites could be tested for the presence of the above microbial lineages and could indicate ongoing deleterious effects and help mitigate damage. While our study focused on specific modes of degradation of polymer composites, it is possible that additional currently unknown mechanisms exist for this process. Future studies should complement genome-based analyses with measurements of activity and isotope-based quantification of the magnitude of degradation of polymer composites.

A recent report describing the state of infrastructure in the U.S. from the American Society for Civil Engineers revealed that current infrastructure in the U.S. is deteriorating rapidly, and failure to act toward improving these would be an issue for the nation’s public safety, economy, and national competitiveness. Fiber-reinforced polymer composite products and systems
can provide cost-effective replacements for rehabilitating or retrofitting existing infrastructure compared to conventional construction materials used in harsh environments. For example, polymer composites can be used for rehabilitating structures made of steel, timber, or concrete at about 5% of their replacement cost. In recent years, there has been an increasing demand for using polymer composites in designing new or rehabilitating existing infrastructure under harsh environments. Given the lightweight nature of polymer composites, they are also excellent candidates for applications in remote regions, for example, like the Arctic. As the Arctic region evolves due to the melting of sea ice, we will encounter novel physical, chemical, and biological factors that are not prevalent in regular environments, and an approach like the one developed here can assist in identifying and modeling durability of polymer composites in the Arctic.

Microbial activity is an important environmental factor in most marine, terrestrial, and coastal environments, where these polymer composites are likely to be used. The five important microbially mediated degradation mechanisms of polymer composites recognized in this study motivates new thinking about how their durability should be modeled. Although the current study focuses on polymer composites with vinyl ester resin, the observations and conclusions are also relevant and beneficial to other resin systems like epoxies and polycarbonates, where acrylate and bisphenol are key compounds. Hence, we propose that microbial interactions with polymer composites should be considered in future modeling of polymer composites developed for long-term durability and life prediction.

**Methods**

**Collection of microbial samples.** To obtain microbe-rich soil samples for biofilm incubation, soil was extracted from a location at Picnic Point near Lake Mendota, WI, USA (Fig. 2). The map showing the location of soil sampling in Fig. 2 was generated using the ggmap package in R version 4.0.2 (ref. 62). The get_map function of the ggmap package was used to obtain the map (maptype = “roadmap” and source = “osm”). Microbial biofilms were collected carefully using sterile techniques from the polymer composite surface and stored frozen for subsequent DNA extraction. Biofilm sampling was performed once every two weeks for 24 weeks, with duplicate samples being taken on all samples, except for weeks 2, 4, and 10, due to inadequate growth of the biofilm. Samples were stored at −80 °C until DNA was extracted, or the samples were otherwise utilized.

**Synthesis of vinyl ester-based polymer composites.** We used 3 K tow (i.e. 3000 filaments per tow) plain weave carbon fiber fabrics procured from FIBREGLAST® (fibreglast.com) as the reinforcing material for fabricating the composite laminates. In addition, we used Hetron 922 vinyl ester resin with 1.25% MEKP (both procured from FIBREGLAST®) as a catalyst, to permeate through the dry carbon fabric and serve as the binding matrix. Mechanical properties of the carbon fabric and vinyl ester resin can be found in our previous study43. Additionally, we measured the average molecular weight of vinyl ester monomer using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) analysis and reported an average molecular weight of $\sim$1200 g mol$^{-1}$ in Supplementary Fig. 7.

**Thermogravimetric analysis.** TGA is a thermal analysis tool used for evaluating the physical and chemical changes in materials as a function of temperature. A material sample is heated at a constant heating rate and the temperature-dependent mass change is established. Samples collected from different environments subjected to TGA usually present temperature-dependent mass change, which is related to the chemical or physical changes due to temperature and specific environmental conditions. Herein, we conducted TGA on polymer composite samples using a Netzsch TG 209 F1 Libra (Netzsch Instruments North America, LLC) setup fit with nitrogen as the purge gas. We used nitrogen gas to maintain an
inert atmosphere in the test chamber to act as a protective gas for our sample and instrumentation. We collected samples from the region close to the surface of the polymer composites, which were periodically collected from the soil samples. After poling, we mounted the specimens on a magnetic disk using epoxy glue and then performed nanoindentation on the specimens. We applied a maximum load of 8000 μN ($P_{\text{max}}$) at a loading rate of 1600 μNs$^{-1}$. This maximum load was maintained for 2 s, after which the specimen was unloaded at the same rate of 1600 μNs$^{-1}$. We performed a minimum of ten indentations on each assessed region for reproducibility. We used the area of each indentation to obtain the hardness and modulus value using Eqs. (1)–(3).

$$\text{Modulus, } E_s = \frac{S}{d^3} \sqrt{\frac{\pi}{3}} A$$

(1)

Indentation depth, $h = \frac{h_{\text{max}}}{2} - 0.75 \times \frac{P}{S}$

(2)

Dissolved organic carbon. DOC is defined as the fraction of total organic content (TOC) in a solution that passes through a 0.45-μm filter. The part remaining on the filter is called particulate organic carbon or non-dissolved organic carbon (POC/ NDOC). We collected solutions from different conditions in this study and filtered them individually through a 0.45-μm syringe filter (25-mm cellulose acetate membrane). We then transferred the solutions into centrifuge tubes/vials (PP, graduated, 50 ml). We centrifuged (Eppendorf 5804R centrifuge) the solutions at a rate of 4500 r.p.m. for 60 s and decanted the top solution after that. We then transferred the filtered and decanted solution to a vial. The vials were baked at 400 °C for sterilization before using them for measurements. We kept the vials serially on the sample holder in addition to three blank vials for measurements with deionized water (electrical resistance—18 MΩ) to obtain a baseline. We then programmed the procedure for each measurement in a GE Siwes autosampler software, where we chose the default reagent process for blank run and chose auto reagent process for unknown runs. We flushed the system (GE Siwes MS310C, USA) with deionized water (electrical resistance—18 MΩ) three times before obtaining each measurement. We measured the DOC concentration in solutions (20 ml) from different conditions using a TOC analyzer (GE Siwes MS310C, USA), and observed that the measurement error was within ±3%.

DNA extraction. DNA was extracted from biofilm samples using a modified version of the protocol from the Qiagen DNeasy PowerSoil Kit (Qiagen, Hilden, Germany). To modify the protocol, homogenization by vortexing was replaced by homogenization with the MP Biomedicals FastPrep-24 5G machine. Two samples whose concentrations were not sufficient for sequencing were concentrated by centrifugation at 10,000 rpm for 30 min on an InfiniPrep 8000 centrifuge (GE Sievers, Madison, WI, USA) at 65.27 °C, Smax = 1. This maximum load was obtained from the peak of the load-depth curve for a Berkovich indenter with the angle $\alpha = 114.2°$.

**Chemical structure analysis.** FT-IR is a chemical analysis tool specially designed for organic materials in order to verify their chemical structure. This analysis uses the principle of the interaction of infrared radiation between the chemical bonds at different frequencies. This interaction induces vibrational excitation of covalently bonded atoms and groups that have a particular energy intensity at a certain frequency. A typical output of FT-IR is a graph of absorbance/transmittance intensity with respect to wavenumber (frequency). A standard FT-IR spectral library is available for all possible chemical bonds that serves as a fingerprint to match a measured infrared radiation (IR) spectra. For example, C=O bonds interact with the IR frequency in a range of 1650 cm$^{-1}$–1800 cm$^{-1}$, which manifests as absorbance/transmittance peak at this frequency range. Changes in chemical bonds are reflected as changes in peak intensity, peak broadening, and peak shifting with respect to the peaks expected at specific frequencies corresponding to a particular type of chemical bonding. In this study, we performed FT-IR to observe changes in the chemical structure of polymer composites before and after exposure to different conditions. We used Bruker Tensor 27 (Thermo Scientific Instrument) equipped with attenuated total reflectance (ATR) to analyze the chemical structure of the composites. The instrument is also equipped with a room temperature deuterium triglycine sulfate (DTGS) detector, mid-IR source (4000–400 cm$^{-1}$), and a KBr beam splitter. This instrument has the capability of scanning IR in the range of 4000–400 cm$^{-1}$ at an increment of 1 cm$^{-1}$, which indicates the degree of linearity of the data obtained by the measurements. Before we placed our sample on ATR sample cell, we ran a background scan with the empty cell using 32 scans at 4 cm$^{-1}$ resolution within the range of 4000–650 cm$^{-1}$ to obtain a baseline. We chose the number of scans and resolutions to obtain a smooth spectrum and used the mid-IR source (4000–400 cm$^{-1}$) to obtain the IR spectrum. We chose a range of 4000–650 cm$^{-1}$ to obtain the IR spectrum as it encompasses all the anticipated chemical bonds of interest in this study. After that, we placed the square-cut specimen on top of the ATR sample cell equipped with a single diamond crystal. After placing the sample on top of the crystal spot, the arm rotated over and turned down to press the sample down onto the diamond crystal face to get better contact. Then, we collected the data using Bruker OPUS Data Collection software using 32 scans at 4 cm$^{-1}$ resolution within the range of 4000–650 cm$^{-1}$. We chose the instrument’s default wide-open aperture settings (∼6 mm) to obtain maximum infrared interaction with our specimen. The aperture is placed in the line of IR beam between the IR sources and interferometer.

**Nano-mechanical measurements.** Nanoindentation is a mechanical measurement tool that enables us to measure mechanical properties such as modulus and hardness in nanoscale. Primarily, load and depth of indentations are measured during nanoindentation. Different shapes of indenters have the ability to measure different nanomechanical responses, for example, Berkovich-type indenter is used for modulus and hardness, cube corner for fracture toughness, spherical cone for scratch, and wedge for three-point bending (R exural) measurements. In this study, we performed nanomechanical measurements in a Hysitron triboindenter T900 equipped with an optical microscope for imaging. We chose Berkovich-type Nano indenter for nanoindentation, which was calibrated using a reference specimen of fused silica and acrylonitrile butadiene styrene (ABS). We performed the nano-mechanical tests as per ASTM standard E2546-15 (ref. 63) on polished cross-sections of composite specimens, which were periodically collected from the soil samples. After polishing the composite cross-section, we used 400–1200 grit SiC metallurgical abrasive grinding paper (Pace Technologies, USA) for fine grind and then polished with 3-μm diamond solution on DACRON II Polishing Cloth (Pace Technologies, USA). Upon polishing, we mounted the specimens on a magnetic disk using epoxy glue and then performed nanoindentation on the specimens. We applied a maximum load of 8000 μN ($P_{\text{max}}$) at a loading rate of 1600 μNs$^{-1}$. This maximum load was maintained for 2 s, after which the specimen was unloaded at the same rate of 1600 μNs$^{-1}$. We performed a minimum of ten indentations on each assessed region for reproducibility. We used the area of each indentation to obtain the hardness and modulus value using Eqs. (1)–(3).

The two metagenome samples from weeks 8 and 18 were assembled independently. Read trimming was performed with Sickle version 1.33 (https://github.com/najoshi/sickle)
using paired-end read trimming with the encoding parameter "1-s anger." Trimmed reads were assembled with SPAdes v3.12.0 (ref. 75) (MetaSPAdes) using the flag -meta and by specifying kmer values of 21, 33, 55, 77, 99, and 127 for assembly. Only scaffolds larger than 1000 bp were considered for downstream analyses. Differential coverage-based genome binning was performed using the metawrap76 pipeline, making use of two binning softwares: metanab (ref. 77) and metabet2 (ref. 77). To extract and consolidate the best bins produced by each binning software, DASTool78 was run on the bins with the flag "-score_threshold 0.4." Genome completeness and contamination were identified using CheckM79 and DASTool. To identify preliminary taxonomy, GTDB-Tk80 was run on obtained MAGs using the classify_wf function. The quality of draft genomes was established using MIMAG standards81. Open reading frames/Proteins were predicted from the assembled scaffolds using the metagenome mode of Prodigal71 (-p meta).

**Concentrated 16 ribosomal protein phylogeny (RP16)** A curated set of publicly available 16 ribosomal protein Hidden Markov Model (HMM) files were utilized to search for ribosomal proteins in the MAGs. All annotated proteins were queried using hmmsearch82 with trusted cutoffs for each HMM. A custom python script was used to sort output tables by score and remove duplicate hits to a single genome by retaining hits with higher scores.

To generate a robust concentrated protein alignment of all 16 extracted ribosomal proteins, a manually curated set of these 16 ribosomal proteins for approximately 3500 reference genomes was obtained and imported along with the ribosomal proteins from the MAGs into Geneious Prime. Ribosomal protein sequences were aligned using MAFFT v7.450 (ref. 77) (Algorithm: Auto, Scoring matrix: BLOSUM62, Gap open penalty: 1.53, Offset value: 0.123). Geneious Prime was used to concentrate the alignments generated for the 16 ribosomal proteins. All alignment columns that had gaps >95% were removed. A preliminary tree was generated with FastTree Version 2.1.11 (ref. 77) run with default parameters. The tree was utilized to identify reference genomes that could be removed with little effect on the phylogenetic placement of the MAGs from this study. This resulted in approximately 1500 reference genomes that were used for the final phylogenetic tree. The final tree was implemented in RAxML-HPC-HPC v.8 (ref. 79) on the CIPRES platform with the following parameters: bootstopping type autoMRE, choose_bootstop bootstop, datatype protein, parsimony_seed_val 12345, -w 8798, -x 8898, -n 8898, -m LGG, -o figtree, -s 8798, -t 8898, -u 8898, -v 8898.

**Calculation of replication rates.** All MAGs from the four most abundant phyla Deltaproteobacteria, Chlorobi, Chlororfez, and CPR/Putichi bacteria were used to estimate replication rates. Trimmed reads were mapped onto genomes using Bowtie2 (ref. 82). Read mapping was conducted on each of the genomes, using the corresponding set of reference sequences (e.g. Genomes from week 8 were mapped with reads from week 8). Shrinksmans Version 0.90 (https://github.com/bichothams/shrinksmans) was used to generate a reduced SAM file containing only reads that mapped to the genome. The sorted SAM files were used as input for iRep53, which was run with default parameters for each genome.

**Calculation of relative abundance/coverage of MAGs.** Genome Coverage of all MAGs was determined by read mapping using Bowtie2. All mapped SAM files were converted to BAM format using samtools. CoverM (https://github.com/wwwood/CoverM) was used to generate read coverage information from the BAM file. An in-house perl script was used to average the depth values for each of the scaffolds within each bin, thereby generating coverage values for each genome bin.

**Determination of metabolic potential.** The metabolic potential of all MAGs was determined using METABOLOC-C. METABOLOC-C was run on each MAG with reads from weeks 8 or 18 depending on the sample that the MAGs originated from (e.g. MAGs from week 8 were used with reads from week 8). METABOLOC-C was used to generate Supplementary Figures 2 and 3. Resultant metabolic charts produced by METABOLOC-C that involved use of the KEGG database were analyzed by referencing KEGG modules to confirm reactions performed by each step.

**Determining microbial mechanisms of polymer composites breakdown.** Several metabolisms proposed to be involved in the degradation of polymer composites and described in 1. Metabolic pathways associated with polymer composite degradation were identified using HMM-based searches against specific databases (Pfam83, KEGG84, custom) and BLAST85 searches using amino acid fasta files (Supplementary Data Table 3 Table 19). For HMM profiles obtained from KEGG, the provided trusted cutoffs were used to limit hits to the MAGs in the study. For HMM profiles obtained from Pfam, an e-value cutoff of 1e−20 was specified to limit hits. An HMM profile was used to query for the presence of an amidase, Amin (EC 3.5.1.4), because it has been shown to be used in the degradation of polyacrylamide86. A set of proteins shown to degrade acrylic, bisphenol A, and methyl ethyl ketone were identified through MetaCyc87, and the corresponding amino acid fasta files were queried against the MAGs using a BLAST search with an e-value cutoff of 1e−20 and a percent identity cutoff of 50%.

**Data availability** All DNA sequences (genomes and sequence reads) have been deposited in NCBI Genbank in the BioProject database with accession code PRJNA615962. NCBI Genbank accession numbers for individual genomes are listed in Supplementary Data 3 Table 7. The authors declare that all other data are available within the article and its Supplementary information files, or from the corresponding authors on request.

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Author contributions
A.B. and M.I. carried out the experiments. A.B., Z.Z., and K.A. conducted the microbial analyses. M.I., A.B., P.P., and K.A. wrote the manuscript. M.A.A. and J.C.N. conducted the molecular weight measurements and contributed toward reviewing and adding valuable information to the manuscript. P.P. and K.A. supervised the project. P.P. and K.A. conceived the original idea. All authors discussed the results and contributed to the final manuscript.

Competing interests
The authors declare no competing interests.

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