Think global, act local: The small-scale environment mainly influences microbial community development and function in lake sediment

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Abstract

The early stages of community development influence longer-term establishment of species, traits, and ultimately ecosystem function. How this process varies with small- and large-scale abiotic and biotic conditions is poorly studied in microbes. Here, we tested how different spatial scales influenced the rate at which taxonomic and functional composition of microbial communities changed over time in lake sediments, and whether these changes occurred synchronously across different environments given the same initial communities. We manipulated the small-scale environment by creating sediments with different terrestrial organic matter (t-OM) quantity and quality, and placing these in two lakes differing in trophic status to vary the large-scale environment. We found that archaeal and bacterial communities, but not fungi, became taxonomically dissimilar over 2 months despite being derived from the same initial leaf material, primarily because of small-scale environmental conditions. Sediment t-OM quantity consistently explained changes in community composition both temporally within mesocosms and spatially between mesocosms in different lakes. Archaea, bacteria, and fungi also varied by up to 10 times in how quickly they changed, providing among the first evidence in the same study system that they respond differently over time to abiotic and biotic conditions. Finally, functional composition was influenced by both small- and large-scale environmental conditions, with genes involved in t-OM decomposition showing some of the largest changes in abundance after 1 yr. Our study highlights that future changes to both sediments and lake waters can modify how sediment microbial communities develop with consequences for important ecosystem functions like carbon cycling.

Understanding how microbial communities develop with time can help improve predictions of ecosystem functioning, such as biogeochemical cycling. Most of what is known about how microbial communities assemble in nature comes from soils, waters, and associations with plants and animals, with much less known about lake sediments (Shade et al. 2013; Thompson et al. 2017; Ortiz-Alvarez et al. 2018). Microbial dynamics in lake sediments are important to study because they have a widespread influence over many ecosystem functions, such as carbon cycling (Tranvik et al. 2009; Heathcote et al. 2015; Drake et al. 2017). Yet the factors influencing microbial composition in lake sediments will differ from soils and waters because they are positioned downslope from sources of microbial colonists, and have different hydrology (e.g., residence time) and unique local environmental conditions (e.g., physicochemical gradients and resource availability)—all factors that influence microbial diversity (Crump et al. 2012; Niño-García et al. 2016). Therefore, sediment microbial communities should develop differently than other systems in response to small- and large-scale abiotic and biotic factors that determine which species establish and persist in a site (Langenheder and Székely 2011).

Little is known about how the rate at which microbial communities develop is jointly influenced by environmental conditions at different spatial scales, especially in lake sediments. For example, large-scale factors, such as climate, may explain much more variation in temporal patterns of community composition than smaller, site-specific factors, such as pH (Kent et al. 2007). The strength of small- and large-scale factors can also vary with time and occur at different rates. For example, large-scale factors, including climate (Lima-Mendoza...
et al. 2015; Delgado-Baquerizo et al. 2018) and disturbance (Shade et al. 2011), can initially determine which species colonize a site (Götzenberger et al. 2012; de Bello et al. 2013). Small-scale factors, such as substrate availability and physico-chemical characteristics (Ruiz-González et al. 2015; Orland et al. 2019), can then act on abundances and co-occurrence patterns (de Bello et al. 2013; Tedersoo et al. 2014). However, there has been no attempt, to our knowledge, to test how different environmental scales influence temporal changes in lake sediment microbial communities.

Biotic interactions simultaneously vary with abiotic conditions (Kraft et al. 2015; Cadotte and Tucker 2017), and thus combine to influence how communities change with time (Kent et al. 2007; Fuhrman and Steele 2008). For example, whether fungi or bacteria are the first to establish in a site may have strong consequences for subsequent species composition. Aquatic saprophytic fungi can decompose some of the most recalcitrant organic compounds from humic substances, subsequently allowing bacteria to colonize humic environments and break down these compounds further if they arrive after the fungi (Grossart and Rojas-Jimenez 2016). Further support for the idea that arrival order influences the rate of change in other lineages comes from evidence that taxa from different lineages interact metabolically. For example, bacterial growth can be promoted by fungal exudates (Pion et al. 2013; Ponomarova and Patil 2015). Few studies have, however, differentiated among bacterial, archaeal, and fungal community changes despite them differing in their metabolic abilities (Fierer et al. 2010; Morriën et al. 2017).

Differences in community assembly can ultimately lead to variation in the important functions performed by microbes in lake sediments. Historically, many studies reported that abiotic and biotic factors influenced compositional turnover and the subsequent activity of lake sediment microbes involved with biogeochemical cycling (e.g., Jones and Simon 1981; Toerien and Cavari 1982; Capone and Kiene 1988; Holmer and Storkholm 2001; Hakulinen et al. 2005). High-resolution genomic tools now offer the potential to understand how the genetic content of these communities varies across different environments and lineages in more detail than ever before (Tringe et al. 2005; Linz et al. 2019). Using such tools, we predicted that communities would diverge fastest with identical t-OM but positioned in different lakes. Using this comparison, we asked which small-scale environmental conditions influenced the rate at which different communities (i.e., different mesocosms) became dissimilar from one another through time. We focused on archaeal, bacterial, and fungal community structure, as well as the connectivity of individual taxa, which can shed light on any biotic interactions underlying compositional changes, for example, facilitation and competition. We predicted that communities would diverge fastest from each other under nutrient-rich conditions, because these provide more opportunities for growth and for colonization by novel species. We also expected different microbial groups (i.e., archaea, bacteria, and fungi) to change at different rates because they respond differently to their abiotic and biotic surroundings. Finally, we asked how the development of communities under different environmental conditions ultimately influenced the potential functioning of lake sediments. We expected those conditions responsible for the greatest changes in taxonomic composition would eventually result in the greatest changes in function.

Materials and methods

Experimental design

Mesocosms with different types of t-OM were placed on the nearshore bottom of two lakes beneath 0.30–0.75 m of water during July 2015. The mesocosms were constructed out of High-density polyethylene (HDPE) containers that measured $50.8 \times 38.1 \times 12.7 \text{ cm}^3$ and were filled with ca. 15 L of sediment to a depth of 8 cm after Tanentzap et al. (2017). Briefly, we added 5%, 25%, and 50% t-OM on a dry-weight basis to 7 kg of
locally sourced inorganic material. The inorganic material consisted of clay, fine sand, and gravel particles (0.063 mm, 0.063–1 mm, > 1 mm diameter, respectively). For each t-OM quantity, material was comprised of either primarily deciduous, coniferous, or mixed litterfall collected from nearby forests, which had been mulched and sorted into < 1 and 1–10 mm diameter fractions. The deciduous treatment contained 66% litterfall by dry-weight mainly from Acer rubrum, Betula papyrifera, Populus tremuloides, and Quercus spp. Coniferous litterfall dominated by Pinus resinosa comprised the remaining material. In the coniferous treatment, the ratios were reversed, with Pinus spp. representing 66% of dry-mass and deciduous litter the remainder. The mixed treatment had equal dry-masses of deciduous and coniferous material. Particle sizes for both inorganic and organic material mimicked natural nearshore lake sediments (Tanentzap et al. 2017). We also homogenized inorganic and organic material because nearby nearshore sediments showed no vertical structuring in the top 8 cm (Tanentzap et al. 2017). Each treatment was then replicated three times, resulting in a total of 3 t-OM quantities × 3 t-OM qualities × 3 replicates (total n = 27 per lake). Mesocosms were arranged in a block design between two sampling bays, submerged in rows, and covered with a 1 mm × 1 mm nylon mesh screen to standardize the percentage of sunlight reaching the sediment surface. After 1 month, we made an 8 cm slit in the center of each screen to collect sediment. Importantly, pore water taken from the surrounding natural lake sediment had statistically indistinguishable pH, dissolved organic carbon (DOC) concentrations, and zooplankton biomass to mesocosms with identical t-OM quantity (i.e., 5% t-OM; Tanentzap et al. 2017), allowing us to extrapolate our findings to field conditions.

Environmental characterization
We submerged the experimental mesocosms in the nearshore region of two small lakes outside of Sudbury, Canada to provide different large-scale environments within the same regional species pool: Lake Laurentian (46°27′30″N, 80°56′0″W; area = 1.57 km²) and Swan Lake (46°21′59″N, 81°3′49″W; 0.06 km²). The different large-scale environments arose because the lakes differed in their overlying water quality. Following Williamson et al. (1999), we classified and hereafter refer to Laurentian and Swan as mixotrophic and oligotrophic, respectively. Lake Laurentian is mixotrophic as it has a total phosphorus (TP) concentration from summer midlake surface grabs of 35.2 μg L⁻¹ and colored dissolved organic matter (CDOM; i.e., absorption coefficient at 320 nm) concentrations of 26 μM. By contrast, Swan Lake is oligotrophic because it has much lower TP and CDOM of 9.3 μg L⁻¹ and 1.5 μM, respectively. Despite these differences, the two lakes are surrounded by similar early successional forest and experience minimal human disturbance. Almost all of the land cover in the Swan catchment (area = 0.2 km²) is mixed-conifer forest (96%), with the remainder covered by granite outcropping and wetland, whereas the larger Laurentian catchment (area = 10.6 km²) is surrounded by less mixed-conifer forest (41%) and more rock (20%) and wetland (10%) (Ontario Ministry of Natural Resources [OMNR] 2009). There is also some low-density suburban development (28%) at the far southwest edge of the Laurentian catchment (i.e., more than 3 km from the center of the lake; OMNR 2009), which is separated by conservation lands.

To characterize the small-scale environment, we sampled pore water from each mesocosm during three periods in 2015: 10–12 August, 07–09 September, and 05–07 October. Pore water was collected from a 3 mL polypropylene syringe secured horizontally immediately beneath the sediment surface prior to filling containers with material. The wall of the syringe that faced the sediment was removed and covered in ca. 250 μm nylon mesh, and the syringe was connected to nylon tubing secured to a float on the lake surface. After purging the nylon tubing, we extracted 45 mL of pore water on each occasion and immediately measured pH with a handheld meter (HI 9126, Hanna Instruments, Woonsocket, RI, U.S.A.). We then filtered 25 mL of each sample through a 0.5 μm glass fiber filter (Macherey-Nagel MN 85/90), which was presoaked in triplicate with MilliQ water, and into a 20 μm glass scintillation vial, which was precalibrated for a pH of approximately 2–3 to avoid the effects of metal quenching on dissolved organic matter (DOM) fluorescence (Spencer et al. 2007). In the lab, we measured two widely used DOM metrics using a Cary 60 UV Vis spectrophotometer and a Cary Eclipse fluorescent spectrophotometer (Agilent Technologies, Santa Clara, CA, U.S.A.). The first DOM metric was the specific UV254 absorbance (SUVA), which is an index of the average aromatic fraction of DOM per unit DOC, itself measured on a Shimadzu TOC-5000A (Shimadzu, Columbia, MD, U.S.A.). Higher SUVA values indicate higher molecular weight DOM that tends to be more difficult for microbes to break down (Sinsabaugh et al. 1997; Lavonen et al. 2015). We also corrected SUVA values for iron, which absorbs UV at a similar wavelength to SUVA and can artificially inflate SUVA measurements (Weishaar et al. 2003; O’Donnell et al. 2012). Total iron concentrations were measured using the FerroVer method on a Hach DR3900 spectrophotometer (Hach-Lange, Loveland, CO, U.S.A.). The second DOM metric was the humification index (HIX), for which higher values indicate less structurally complex DOM and increased humic substance content (Fellman et al. 2010).

Microbial communities
Taxonomic composition was characterized from surface sediment grabs (ca. top 5 cm) collected from all mesocosms during each of the three sampling periods in 2015 (total n = 54 mesocosms × 3 dates = 162). We filled a 60 mL sterile trowel with sediment from the center of the mesocosm and immediately placed the sediment into individual sterile sample bags that were freeze-dried at −20°C to stabilize the communities (Miller et al. 1999). DNA was also extracted in triplicate from the original deciduous and coniferous t-OM (i.e., prior to any mixing between them or with inorganic
material). 16S and ITS primers targeting archaea, bacteria, and fungi were used to construct sequencing libraries for all samples after all environmental DNA was extracted (see Supporting Information Text S1 for further details). We used universal prokaryotic primers and universal eukaryotic primers rather than more universal primers targeting all microbial groups as more taxa will be missed as the captured phylogeny is broader in spectrum (Wurzbacher et al. 2017). Libraries were then sequenced on an Illumina MiSeq (600 cycles, paired-end) at an average (± SE) read depth of 15,781 (± 514) and 26,573 (± 1879) reads for the 16S and ITS sequencing runs, respectively.

We inferred amplicon sequence variants (ASVs) present in each sample from the raw sequences and taxonomically annotated them using DADA2 separately for each of the 16S and ITS data sets (Callahan et al. 2016; Supporting Information Text S1). Unlike operational taxonomic units (OTUs), which are arbitrarily determined by a 97% threshold sequence similarity, each ASV is defined as a unique sequence thanks to a method that controls for amplification and sequencing errors (Callahan et al. 2016). ASVs therefore represent unique microbial taxa, and yield more accurate and reproducible amplicon data across studies than OTUs (Callahan et al. 2017). From all samples, we removed the ASVs that were present in three negative water-only controls with a relative abundance of > 1% (42 out of 35,674 ASVs for the 16S data set and 5 out of 6952 ASVs for the ITS data set). As we were interested in tracking how the bulk of the communities changed with time, we focused our analysis on the 1% most abundant taxa as per Flores et al. (2012). The resulting 889 and 625 taxa for the 16S and ITS data sets together comprised on average (± SE) 70% (± 1%) and 94% (± 0.01%) of the total reads in each sample, respectively. We controlled for differences in the number of reads per sample due to sequencing biases with a widely applied variance stabilizing transformation (Dillies et al. 2013; McMurdie and Holmes 2014) using the R package DESeq2 (Love et al. 2014). While there is no perfect method to normalize sequencing data, DESeq2 is currently among the most widely recommended approaches to allow between-sample comparisons (Hugert and Andersson 2017; Knight et al. 2018). It is also considered one of the better normalization methods for libraries that are relatively large and of similar size, like ours (Weiss et al. 2017). Changes in abundance would have been impossible to estimate had we used a more traditional rarefaction method, as increases in the relative abundance of one taxa would systematically lead to the decrease in the relative abundance of others. For these reasons, all downstream analyses were performed on the DESeq-transformed data. The raw sequences were deposited in EBI under the project accession number ERP110084.

We returned to our sites after 1 yr to characterize microbial functions using shotgun sequencing. We acquired an additional surface sediment grab in August 2016 to compare with the previously acquired September 2015 samples. Sequencing libraries were prepared from both dates with 1 ng of genomic DNA per sample using the Nextera XT DNA Sample Preparation Kit and dual-barcoding with Nextera XT Indexes (Illumina, San Diego, CA, U.S.A.), and then sequenced on an Illumina NextSeq (300 cycles, paired-end; see Supporting Information Text S1 for further details). Raw sequences were processed following the European Molecular Biology Laboratory-EBI pipeline version 3.0 (Mitchell et al. 2016) and summarized with Gene Ontology (GO) terms. The sequences were deposited in EBI under the project accession number ERP019980.

Do microbial communities diverge faster through time because of differences in lake or sediment conditions?

We first assessed how quickly microbial communities diverged through time from those present on the original mesocosm t-OM, that is, leaf material. We constructed matrices of samples by ASVs for each microbial group (i.e., archaea, bacteria, and fungi). We then used the Bray-Curtis dissimilarity index between all pair-wise combinations of samples. This index, as formulated in the R package vegan v. 2.5.2, sums the absolute difference in relative abundance for each ASV between two samples and divides this value by the summed relative abundances of all ASVs in the two samples (Oksanen et al 2016). A value of 0 indicated communities were entirely similar while a value of 1 indicated communities were entirely different, that is, no overlapping taxa (Bloom 1981). As the index is based on relative abundances, it is invariant to differences in absolute abundance (Jost et al. 2011). To test whether the rate at which communities diverged from the original leaf material varied among lakes, we fitted separate linear models to the Bray-Curtis values for each microbial group (i.e., bacteria, archaea, and fungi). The models included sampling day, lake, and an interaction term between sampling day and lake as predictors. For all linear models, we corrected SEs as in Cleasby and Nakagawa (2011) with the coeftest function in the lmtest R package (Zeileis and Hothorn 2002) because the variance across treatment levels was not constant.

We also tested whether compositional differences were associated with sediment pore water within each lake. We first visualized taxonomic composition in all the mesocosms with nonmetric multidimensional scaling (NMDS) ordinations using the matrices of samples by ASVs as inputs. Separate ordinations were performed for each microbial group (i.e., bacteria, archaea, and fungi). We then used the envfit function in the vegan R package (Oksanen et al. 2016) to correlate the NMDS scores with pH, and log-transformed values of SUVA, DOC, and HIX. Significance was determined using 999 permutations of the raw data.

Do microbial communities become dissimilar from each other faster because of differences in sediment or lake conditions?

We tested whether the rate at which spatial differences in microbial communities accumulated with time differed with
sediment and lake conditions. We calculated the Bray-Curtis dissimilarity on each sampling date between mesocosms that received identical t-OM treatments and were in the same position in our experimental block design but located in different lakes. We then used a linear model to test how dissimilarity changed over time with different quantities and qualities of t-OM. We added interaction terms between the sampling day and each of t-OM quantity and quality, along with their main effects. Sampling bay was also included to account for the blocking design of our experiment. Separate models were fitted for each microbial group.

We also asked whether individual ASVs occurred more with each other over time because of sediment or overlying lake conditions. The corresponding analysis effectively required counting how many times each ASV co-occurred with all other ASVs. To analyze this value in relation to our experimental design, we had to stratify the counts by each level of each experimental treatment. Therefore, we created separate co-occurrence networks at the start (August 2015) and end (October 2015) of the taxonomical profiling for each t-OM quantity, t-OM quality, and lake using the igraph R package (Csárdi and Nepusz 2006). A total of 16 networks were generated, from which we estimated the number of degrees (i.e., co-occurrence among each taxa, or connectivity; Tylianakis et al. 2010) for each ASV. As the minimum number of ASVs in a microbial group was 44 (i.e., for archaea), we only constructed networks for the 44 most abundant ASVs in each microbial group to keep the maximum number of degrees consistent. We then measured the difference in the number of degrees for each ASV between October and August for each t-OM quantity, t-OM quality, and lake. We assessed how the number of degrees per ASV changed over time using a linear model that included either t-OM quantity, t-OM quality, or lake as a predictor and allowed these responses to vary with microbial group.

**Do microbial functions change with time because of sediment or lake conditions?**

We tested whether temporal change in the microbial functions present in each mesocosm varied along the same environmental gradients as taxonomic composition. First, we normalized read counts for all annotated GO terms (n = 2765) using a variance stabilizing transformation with the DESeq2 R package (Love et al. 2014). We then fitted linear models to the counts for each GO term, allowing sampling date to interact with each treatment factor (lake, t-OM quantity, and t-OM quality) and including all main effects. The proportion of variance in each GO term explained by each factor, as calculated using type II sums of squares, was extracted with the variancePartition R package (Hoffman and Schadt 2016). For each treatment level, we then calculated the percent of functional genes that significantly changed over time and tested whether this percentage was correlated with the amount of taxonomic change in the respective treatments, as calculated from the absolute difference in Bray-Curtis values between August and October 2015. We also estimated the log2-fold change of all GO terms between the start and end of the experiment by fitting separate negative binomial generalized linear models to normalized read counts for each treatment factor and its interaction with date using the DESeq function in DESeq2. The univariate models allowed us to test for differential abundance of individual GO terms between time periods for each factor level with a Wald test, adjusted for multiple comparisons (Love et al. 2014).

**Results**

**Drivers of taxonomic divergence within mesocosms**

We found that temporal changes in community composition differed across the three microbial groups that we characterized. Both archaeal and bacterial communities taxonomically diverged from the original leaf community over the course of our experiment, but at different rates ($t_{158} = 22.56, p < 0.001$ and $t_{158} = 2.01, p < 0.05$, respectively; Fig. 1). Archaea, which only comprised 44 taxa after filtering to the 1% most abundant 16S ASVs, diverged a magnitude faster than bacteria, which represented 845 taxa (Fig. 1). While archaeal and bacterial communities became increasingly dissimilar over time in each lake, fungal communities did not differ temporally or between lakes (Supporting Information Table S1). Only archaea differed in their dissimilarity from the starting leaf material between the mixotrophic and oligotrophic lakes (Supporting Information Table S1). Across lakes, archaeal and bacterial sediment communities were driven away from the original leaf community as pH, SUVA, and HIX increased (respectively $R^2 = 0.33, p = 0.001$, $R^2 = 0.06, p = 0.009$, $R^2 = 0.14, p = 0.001$, for archaea; and $R^2 = 0.16, p = 0.001$, $R^2 = 0.07, p = 0.007$, $R^2 = 0.10, p = 0.001$ for bacteria; Supporting Information Figs. S1, S2). DOC was not associated with changes in archaeal and bacterial community composition and fungal community composition did not vary along any of the environmental gradients (Supporting Information Table S2).

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**Fig. 1.** Archaeal and bacterial communities diverged from the original leaf community. Points are mean ± SE for divergence rates from the original leaf community for each microbial group. Rates significantly different from 0 denoted by *$p \leq 0.05$, ***$p \leq 0.001$.**
We found that microbial communities became taxonomically dissimilar between lakes at different rates. Consistent with our previous findings (Fig. 1), archaeal communities became taxonomically dissimilar from one another about an order of magnitude faster than bacteria or fungi (Fig. 2; Supporting Information Table S3). These differences were not driven simply by changes in abundance, as archaeal ASVs increased with time by a relatively larger margin than the difference among microbial groups in their rates of community change (i.e., compare Fig. 2 with Supporting Information - Table S4). Bacterial ASVs also became more abundant with time (Supporting Information Table S4), suggesting differential levels of species turnover among microbial groups in addition to abundance contributed towards differences in rates of community change. Fungi, however, showed no detectable change in community composition between the two lakes (Fig. 2).

The rate at which identically treated mesocosms located in different lakes taxonomically diverged was influenced by the t-OM treatments. Changes occurred most strongly with increasing t-OM quantity (Supporting Information Table S3). Archaeal communities in the 50% t-OM treatment diverged about one-third-times faster than those in the 5% t-OM treatment (Fig. 3a). The effect was much stronger in bacterial communities, which diverged almost three-times faster in the 50% t-OM quantity treatment than in the 5% t-OM treatments (Fig. 3b). Although the absolute effects may seem small, they were calculated over 2 months and only involved the 1% most abundant taxa. By contrast, we found that communities diverged between lakes at similar rates across the different t-OM quality treatments (Supporting Information - Table S3). Fungal communities again showed no changes with time across the t-OM treatments (Supporting Information Table S3).

Connectivity between taxa increased similarly with time in both lakes when averaged across microbial groups and experimental treatments. However, taxa became more interconnected by the end of our experiment in the 5% and 25% t-OM quantity and mixed t-OM quality treatments (Fig. 4; Supporting Information Table S3).
Information Table S5). Taxa in the 5% and 25% t-OM quantity treatments gained on average 6.30 and 7.65 degrees over 2 months ($t_{387} = 3.93, p \leq 0.001$ and $t_{387} = 4.77, p \leq 0.001$, respectively). Connectivity also increased in mixed t-OM treatments by 9.45 degrees on average over 2 months as compared with 3.52 and 7.29 extra connections in coniferous and deciduous treatments, respectively ($t_{387} = 2.31, p = 0.021$; $t_{387} = 6.22, p < 0.001$, and $t_{387} = 4.80, p < 0.001$; respectively).

![Graph showing changes in microbial connectivity](image)

**Fig. 4.** Microbial connectivity changed with time. Taxa co-occurred more after 3 months in (a) all t-OM quality treatments, (b) lower t-OM quantities, and (c) both lakes. We calculated degrees as the number of co-occurrences between each ASV and all others for the 44 most abundant taxa in each microbial group. Points are estimated marginal means ± 95% CIs for each treatment level averaged across microbial groups. Identical letters indicate treatment levels that were not significantly different from one another. Changes significantly different from 0 denoted by *$p \leq 0.05$, **$p < 0.001$.

![Graph showing functional gene abundance](image)

**Fig. 5.** Temporal changes in functional gene abundance within mesocosms. (a) Percentage of variation across 2765 annotated GO functions explained by t-OM quantity, quality, lake identity, and sampling year. Boxes show interquartile range with points denoting medians. Lines extend 1.5-times the interquartile range, with polygons showing frequency distribution. (b) Heat map for 5% of functions with the most statistically significant ($\alpha = 0.05$) difference in log2-fold abundance between the start and end of the experiment. Colors are changes for each treatment level at mean of all other levels.
Functional changes within mesocosms

Different factors were associated with changes in functional genes after 1 yr vs. those associated with changes in taxonomic composition after 3 months. More variation in GO terms was explained by sampling year (mean: 16%, 95% confidence interval [CI]: 0.01–57%) than any other factor (Fig. 5a). By contrast, temporal changes in individual functions were weakly and similarly influenced by both large- (lake identity) and small-scale environmental conditions (t-OM quantity and quality). All three experimental factors explained less than 6% of variation, on average (95% CI: < 0.01–29%; Fig. 5a). Communiti es where taxonomic composition changed most rapidly early in assembly consequently did not consistently show the largest overall functional changes after 1 yr of development (Spearman’s correlation for archaea, bacteria, and fungi: $\rho = -0.32, p = 0.441$, $\rho = -0.06, p = 0.885, \rho = -0.29, p = 0.481$; Supporting Information Table S6). These results were, however, somewhat unsurprising, as most annotated functions were unrelated to t-OM decomposition and so should have been invariant across the experimental design. More importantly, many of the functions that showed the strongest temporal increases were those associated with t-OM decomposition, for example, methanogenesis, extracellular enzymes, and polysaccharide catabolism (Fig. 5b). Relatedly, functions involved in t-OM decomposition, nitrogen cycling, and methanogenesis had a large proportion (i.e. $\geq 75\%$) of their variation explained by our study design, including different temporal changes among the experimental treatments (e.g., glutamate dehydrogenase and coenzyme-B sulfoethylthiotransferase; Supporting Information Table S7). Functions showing the largest decreases were generally involved in microbial metabolism (Fig. 5b).

Discussion

Our study presents evidence that small- as opposed to large-scale environmental conditions have more influence over the rate at which communities of archaea and bacteria develop and function in sediments of two small boreal lakes. These findings advance previous work because they document how the temporal dynamics of microbial communities in lake sediments differ with environmental scale and provide among the first evidence that major microbial groups assemble differently across time and space when measured in the same study. The implications of these findings are also far-reaching. The weak effect of trophic status suggests ongoing changes to water columns, such as from eutrophication and brownification (Leech et al. 2018), may have little effect on compositional dynamics of sediment microbes unless they also influence sedimentation of coarse OM. This interpretation is consistent with observational evidence that the abundance rather than presence of microbial taxa is limited by physicochemical gradients and resource availability in sediments (Orland et al. 2019). Sediment microbes may instead be responding to fine-scale variation in deposition of t-OM (e.g., Chmiel et al. 2016), and consequently its geochemistry. Studies that upscale microbial processes to entire basins therefore need to sample multiple sites and consider sediments differently from the water column to capture this variability.

Temporal and spatial changes in microbial communities depend on sediment conditions

Temporal divergence of mesocosms from taxonomic communities initially present on t-OM was most strongly influenced by small-scale environmental conditions. Although divergence rates did not differ between lakes, changes more closely tracked humification and pH of sediment pore water. These results advance previous studies that have shown both sediment pH and humification influence microbial community composition at single time points (Ruiz-González et al. 2015; Amaral et al. 2016; Fitch et al. 2018; Tripathi et al. 2018). One explanation for the importance of humic substances is that they may provide favorable conditions for microbial growth by acting both as electron donors and acceptors (Hessen 1992; Lovley et al. 1996; Torres et al. 2011). One caveat of our study is that we focused on changes in the most abundant ASVs. Rare taxa may also contribute to community changes over time (Shade et al. 2014). Importantly, our findings suggest that rates of microbial community change—specifically archaea and bacteria—will accelerate in the near future with increased burial rates of organic carbon from anthropogenic activities (Heathcote et al. 2015).

Mesocosms diverged faster from one another where they possessed higher t-OM concentrations (i.e., different small-scale environment) rather than because they were in different lakes. Despite its high humic and lignin content, t-OM is bioavailable to microbes (Guillemette and del Giorgio 2011; Lapiere et al. 2013) and can be primarily allocated to biomass (Guillemette et al. 2016; Fitch et al. 2018). Consequently, the increase in abundances associated with higher t-OM can result in compositional changes and faster divergence rates, especially in the early stages of community development (Fierer et al. 2010; Muscarella et al. 2016). The quantity and source diversity (i.e., mixed quality) of t-OM also increased connectivity among ASVs. Thus, in addition to accelerating divergence rates between communities, small-scale environmental conditions simultaneously strengthened co-occurrence networks within communities, thereby reinforcing the differences between communities. Our results are important considering that metabolic dependencies that form between taxa (Zelezniak et al. 2015) may sustain these differences and lead to long-lasting co-occurrence patterns with consequences for ecosystem functioning.

Small-scale environmental conditions likely reflected the combined effects of abiotic and biotic (e.g., competitive) interactions. Disentangling which of these two processes was most important is, however, challenging without experimentally controlling community membership (Kraft et al. 2015), which is impractical in highly diverse, natural microbial communities. Nonetheless, we found support for two of the three lines of evidence that Cadotte
and Tucker (2017) argue would be required to implicate abiotic conditions as determining species survival and persistence at a given locale. These observations were that some communities had very different phenotypes than others, and that the divergence between communities varied along clear environmental gradients. Our study was not designed to test the third line of evidence, which is that the environmental conditions where species peak in abundance should be nonrandomly related to their traits (Cadotte and Tucker 2017). Studies are beginning to address this question with metagenome-assembled genomes, building on reductions in the cost of high-coverage sequencing technology and advances in bioinformatics techniques. For example, methanogens, whose traits are phylogenetically conserved within archaea, reach maximal abundance as permafrost peatlands thaw (Woodcroft et al. 2018), suggesting that abiotic gradients are directly important for structuring these communities. Such findings can ultimately help scale microbial processes to ecosystem functions (Linz et al. 2018).

**Group-specific variation in community change**

Microbial groups varied by up to 10 times in how quickly they changed and, as they are characterized by different traits, such variation could have far-reaching consequences for ecosystem functioning. On average, communities of archaea—the main organisms able to produce the potent greenhouse gas methane (Zheng et al. 2018)—changed 10 times faster than fungi and bacteria regardless of the environmental conditions. Considering that archaea only represented 5% of all the taxa in our 16S data set, we were surprised to find that two archaeal methanogens were the 2nd and 8th most abundant ASVs (both Methanobacterium spp.; Supporting Information Table S8). Lakes release 6–16% of global methane emissions (Borrel et al. 2011), so understanding why these communities changed so rapidly is important for predictions of whole-lake C cycling. One potential explanation may be that archaea were in much lower abundances on the starting material, so may have been subjected to less intraspecific competition as methane precursors became available after flooding, (partial) anoxia, and anaerobic breakdown. Individual archaean taxa could therefore grow rapidly, as reflected by their increased abundance with time.

We also found that the taxonomic composition of archaean and bacterial but not fungal communities changed with time. Such differences have previously been attributed to high competitive ability and antibiotic production in fungi that allow populations to persist and limit compositional turnover (Bahram et al. 2018; Yakimovich et al. 2018). Fungi can also use the complex carbon substrates that comprise plant cell walls better than bacteria, especially in high nutrient conditions (Koranda et al. 2014), such as found in the mixotrophic lake. Finally, arrival order may also help explain these differences. Lake fungal communities are partly comprised of terrestrial fungi from imported leaves (Bärlocher and Boddy 2016) and these taxa usually dominate the initial stages of plant litter decomposition (Kuehn 2016). It is therefore likely that fungi that were better adapted than bacteria to breaking down t-OM were already present on the original leaf material, allowing them to colonize the sediment first and prioritize its resources, thereby preempting later arrivals from establishing. Such “priority” effects have been found in fungal (Dickie et al. 2012) and bacterial communities (Rummens et al. 2018), but not yet between groups.

**Functional consequences of environmental control over community composition**

Our results suggest that the functional potential of sediment microbial communities changed mostly independent of taxonomy despite some overlap in the importance of small-scale environmental conditions. Functional composition can be decoupled from taxonomy if metabolic functions are widely dispersed across clades or have different roles in different organisms (Louca et al. 2016). For example, in our metagenomics data, functional genes and associated electron carrier enzymes involved in methanogenesis have also been associated with anaerobic methanotrophic clades (Hallam et al. 2003). This association would weaken covariation between changes in archaean communities and genes involved in methanogenesis. Similarly, glutamate dehydrogenase is ubiquitous across the Tree of Life and forms an important part of assimilatory pathways in the N cycle (Cabello et al. 2004). Changes in the abundance of this function would again be unrelated to changes in the taxonomic composition of communities. Nonetheless, we did find that some functions associated with t-OM reflected shifts in taxonomy and community structure. For example, the steep increase in archaean abundances and rapid rate of change in archaean community composition was mirrored by an increase in methanogenesis. Early changes in community development may therefore have consequences for functions closely tied to taxonomy. Analyses that test specific hypotheses around the metabolic potential and expression of functional pathways and their responses to environmental gradients of interest, rather than using data-driven approaches to detect these associations as we do here, may only strengthen the link between function and taxonomy. More generally, our study shows that modifying local environmental conditions early in the development of microbial communities can have consequences for their taxonomic composition and the important functions they perform.

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Conflict of Interest
None declared.