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The Rhizosphere Responds: Rich Fen Peat and Root Microbial Ecology after Long-Term Water Table Manipulation

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ABSTRACT Hydrologic shifts due to climate change will affect the cycling of carbon (C) stored in boreal peatlands. Carbon cycling in these systems is carried out by microorganisms and plants in close association. This study investigated the effects of experimentally manipulated water tables (lowered and raised) and plant functional groups on the peat and root microbiomes in a boreal rich fen. All samples were sequenced and processed for bacterial, archaeal (16S DNA genes; V4), and fungal (internal transcribed spacer 2 [ITS2]) DNA. Depth had a strong effect on microbial and fungal communities across all water table treatments. Bacterial and archaeal communities were most sensitive to the water table treatments, particularly at the 10- to 20-cm depth; this area coincides with the rhizosphere or rooting zone. Iron cyclers, particularly members of the family *Geobacteraceae*, were enriched around the roots of sedges, horsetails, and grasses. The fungal community was affected largely by plant functional group, especially cinquefoils. Fungal endophytes (particularly *Acephala* spp.) were enriched in sedge and grass roots, which may have underappreciated implications for organic matter breakdown and cycling. Fungal lignocellulose degraders were enriched in the lowered water table treatment. Our results were indicative of two main methanogen communities, a rooting zone community dominated by the archaeal family *Methanobacteriaceae* and a deep peat community dominated by the family *Methanomicrobiaceae*.

IMPORTANCE This study demonstrated that roots and the rooting zone in boreal fens support organisms likely capable of methanogenesis, iron cycling, and fungal endophytic association and are directly or indirectly affecting carbon cycling in these ecosystems. These taxa, which react to changes in the water table and associate with roots and, particularly, graminoids, may gain greater biogeochemical influence, as projected higher precipitation rates could lead to an increased abundance of sedges and grasses in boreal fens.

KEYWORDS peatland, carbon cycling, methanogen, bacteria, fungi, archaea, in situ, trace gas, vegetation, boreal ecosystems, climate change, hydrology, iron, plant functional group, plant functional type, root, subarctic

Northern high latitude ecosystems are disproportionately affected by climate change; precipitation and temperature regime shifts are occurring at a higher rate there than in other areas of the world (1, 2). Peatlands, which store over 500 gigatons of soil carbon (C) (3), are particularly susceptible to climate-induced alterations in water table and vegetation (4–8)—both of which drive C cycling, including the production and emission of the greenhouse gases carbon dioxide and methane (9–19). Of the peatland types, carbon cycling in rich fens is especially sensitive to plant community

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shifts (20), which are often driven by the water table position (6). One of the many reasons that plants are effective drivers of biogeochemistry is their intimate and complex relationship with microorganisms, which are understudied in northern rich fens (21–24) and are directly involved in carbon transformation processes, including methanogenesis.

Bacteria, archaea, and fungi comprise the microbial communities that reside in northern fens and soils worldwide. Bacterial and archaeal phyla commonly present include the *Proteobacteria*, *Acidobacteria*, *Actinobacteria*, *Chloroflexi*, *Planctomycetes*, *Bacteroidetes*, *Verrucomicrobia*, and *Euryarchaeota* (25, 26), whereas fungi are generally enriched in the Ascomycota, especially the order Helotiales (27). Fungal dynamics are largely driven by plant functional groups in other peatland ecosystems (28, 29), but there are few studies describing plant-microbial relationships in subarctic rich fens.

Dominant vascular plant functional groups in subarctic rich fens include sedges, grasses, horsetails, and marsh cinquefoil. Sedges have sparse but deep roots, with spongy aerenchymatous tissue capable of transporting air between the atmosphere and rhizosphere. Grasses can form large, dense root balls and may contain facultative aerenchymatous tissue, although it is not well documented if grasses in our study area (such as *Calamagrostis* spp.) have this trait (30). Both sedges and grasses are known to produce root exudates, a trait which can stimulate microbial productivity (31). Horsetails are opportunistic, hardy, and ancient plants containing hollow, segmented stems and rhizomes that run below the peat surface. Previous work suggests gases are transported between the peat and the atmosphere via these hollow stems (32), likely via diffusion (33). Marsh cinquefoil is a shallow-rooting (generally 5 to 10 cm deep), laterally spreading rhizomatous shrub with no evidence of aerenchyma. The diversity of root structure, aeration, and inputs to the soil of these four distinct fen plants (32) could drive differences in root-associated microbial communities. For instance, the enrichment of methanogens via the addition of readily degradable carbon around graminoid roots (34) or an enrichment of microbes associated with the redox cycling of elements such as iron via the plant-mediated aeration of peat could be expected.

In this study, our broad objective was to describe the microbial community of a subarctic rich fen and its dominant plant rhizospheres. Our experimental objectives were to identify how plant functional groups and water table dynamics (i) independently affect the microbial community and (ii) interact to influence microbial activity in boreal rich fens and to describe the collective impacts on carbon cycling, particularly methane production.

To address aspects of the water table-plant functional group effect in tandem, we isolated microbial DNA from peat and plant fine root systems from a long-term experiment composed of three water table treatments (raised, lowered, and control) in a rich fen in interior Alaska, which was initiated in 2004. The *in situ* water table experiment had already caused plant community shifts over time, resulting in fewer sedges in the lowered water table (drought) treatment (6) and less leaf area in general, specifically in relative abundance of sedges, in the lowered water table treatment (35). This provided an opportunity to study the long-term consequences of climate-driven changes on microbial communities in a field setting. We hypothesized that the bulk peat from each of the three water table treatments would contain distinct microbial community compositions, influenced by depth (0 to 10 cm, 10 to 20 cm, 30 to 40 cm, and 60 to 70 cm). Specifically, the oxidative nature of the lowered water table and roots capable of aerating the rhizosphere were hypothesized to enrich biogeochemical cyclers of redox-active elements such as iron. We additionally hypothesized that the microbial communities associated with the roots of four dominant rich fen plants—sedges (*Carex atherodes* Spreng.), grasses (*Calamagrostis* sp. Michx), horsetail (*Equisetum fluviatile* L.), and cinquefoil (*Comarum palustre* L.)—would differ by plant functional group and long-term water table manipulation.

RESULTS

Community response to water table treatment, plant functional group, and depth. The microbial community structure for bulk peat and plant functional group differed by water table treatment, depth, and plant functional group (Tables 1 and 2, Fig. 1). Diversity was largely influenced by depth (see Table S1 in the supplemental material).

TABLE 1 PERMANOVA results of community composition responses to water table treatment, depth, and plant functional group (roots) on prokaryote and fungal communities[a]

	F (df) and *P*			
Model	**WT**	**Depth**	**WT × depth**	**Core (WT)**
Prokaryote bulk peat	2.96 (2, 9.17), **0.001**	26.89 (3, 26), **0.001**	1.31 (6, 26), 0.138	0.72 (9, 26), 0.943
Fungi bulk peat	1.60 (2, 9.9), **0.025**	3.27 (3, 14), **<0.001**	0.99 (6, 14), 0.500	1.15 (9, 14), 0.192
Prokaryote roots	1.80 (2, 23), **0.031**	6.12 (3, 23), **<0.001**	1.19 (6, 23), 0.210	
Fungi roots	2.50 (2, 23), **<0.001**	5.45 (3, 23), **<0.001**	1.96 (6, 23), **<0.001**	

[a]WT, water table treatment; roots, plant functional group roots; core, random effect of core replicates; *P*, probability; df, degrees of freedom (between-group value, within-group value); *F*, F-statistic. Boldface indicates significant differences.

Both the prokaryote and fungal community compositions in the bulk peat were affected by water table treatment and depth, but not their interaction (Table 1). The compositions of plant functional group root prokaryote and fungal communities varied by plant functional group and water table treatment. However, there was no plant functional group × water table treatment interaction in the prokaryote community, whereas fungal composition did exhibit an interaction (Table 1). The greatest numbers of significantly different operational taxonomic units (OTUs) between treatments were found at the 10- to 20-cm depth between water table treatments and between bulk rooting zone peat (0- to 10-cm and 10- to 20-cm depth), cinquefoil, and the other plant functional groups ($P < 0.01$, differential abundance) (Tables S2a and S2c).

Community change included a decrease of the *Proteobacteria* and an increase in the *Chloroflexi* relative abundances with depth. Many *Betaproteobacteria* taxa had greater relative abundances in the raised water table treatment, including *Nitrosomonadaceae*,

TABLE 2 Pairwise community composition (PERMANOVA) results for 16S and ITS across water table treatments and plant functional groups[a]

	Community composition					
	16S			**ITS**		
Pair tested	***t***	***P***	**df**	***t***	***P***	**df**
Bulk peat - water table treatment						
Control - lowered	1.62	**0.02**	6.14	0.99	0.46	6
Control - raised	2.03	**0.03**	6	1.20	**0.03**	7.48
Lowered - raised	1.42	**0.05**	6.22	1.37	**0.05**	7.94
Bulk peat - depth						
0–10 cm to 10–20 cm	3.48	**<0.01**	8	1.07	0.38	3
0–10 cm to 30–40 cm	3.84	**<0.01**	8	1.97	0.05	3
0–10 cm to 60–70 cm	6.62	**<0.01**	8	1.90	0.06	3
10–20 cm to 30–40 cm	3.76	**<0.01**	9	1.17	0.35	2
10–20 cm to 60–70 cm	8.39	**<0.01**	9	2.14	**0.02**	4
30–40 cm to 60–70 cm	4.78	**<0.01**	9	Insufficient data		
Root communities - water table treatment						
Control - lowered	1.57	**0.03**	15	1.47	**<0.01**	15
Control - raised	1.28	0.08	16	1.61	**<0.01**	16
Lowered - raised	1.25	0.15	15	1.63	**<0.01**	15
Root communities - plant functional group						
Grass - sedge	2.18	**<0.01**	11	1.15	**0.02**	11
Grass - horsetail	2.19	**<0.01**	12	2.12	**<0.01**	12
Grass - cinquefoil	2.50	**<0.01**	12	2.46	**<0.01**	12
Sedge - horsetail	2.09	**<0.01**	11	2.54	**<0.01**	11
Sedge - cinquefoil	2.59	**<0.01**	11	2.77	**<0.01**	11
Horsetail - cinquefoil	2.81	**<0.01**	12	2.55	**<0.01**	12

[a]*t*, Student's *t* test value; *P*, probability; df, degrees of freedom. Boldface indicates significant results.

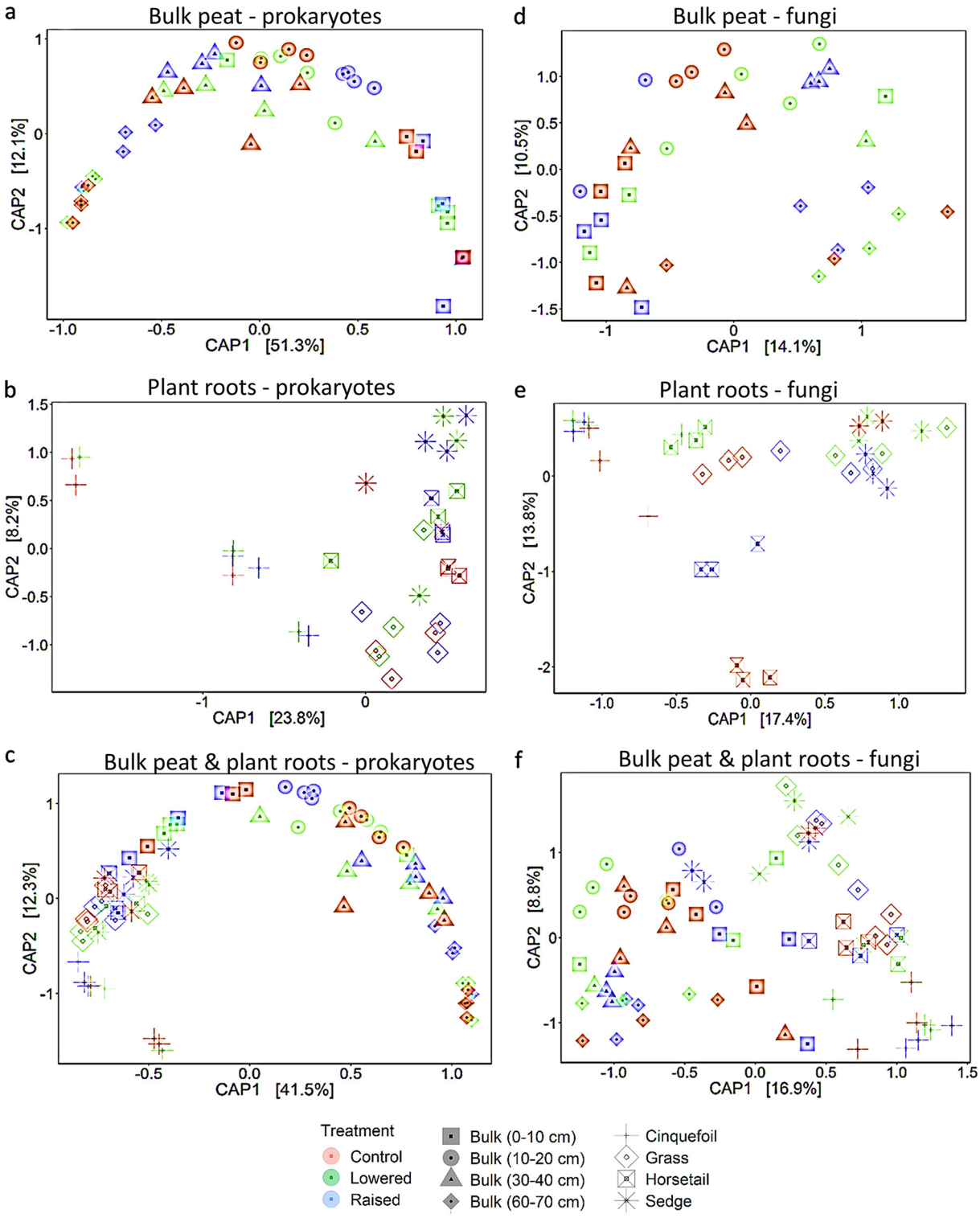

FIG 1 Canonical analysis of principal coordinates (CAP) using Bray-Curtis dissimilarity. (a to f) Ordinations are constrained by the interaction of water table treatment and either depth (a and d) or plant functional group (b and e) and together (c and f) in prokaryote and fungal communities. We acknowledge an arch effect (98) in the prokaryote bulk peat ordinations driven by depth and have left it as is for visualization only.

Comamonadaceae, *Methylophilaceae*, *Neisseriales*, *Rhodocyclales*, and *Uliginosibacterium*. (Fig. 2, Table S3). Fungal communities in the bulk peat showed fewer differences with depth than prokaryotes but tended to be most affected by the raised treatment relative to the other two treatments (Table 2). Pairwise analyses show that fungal community

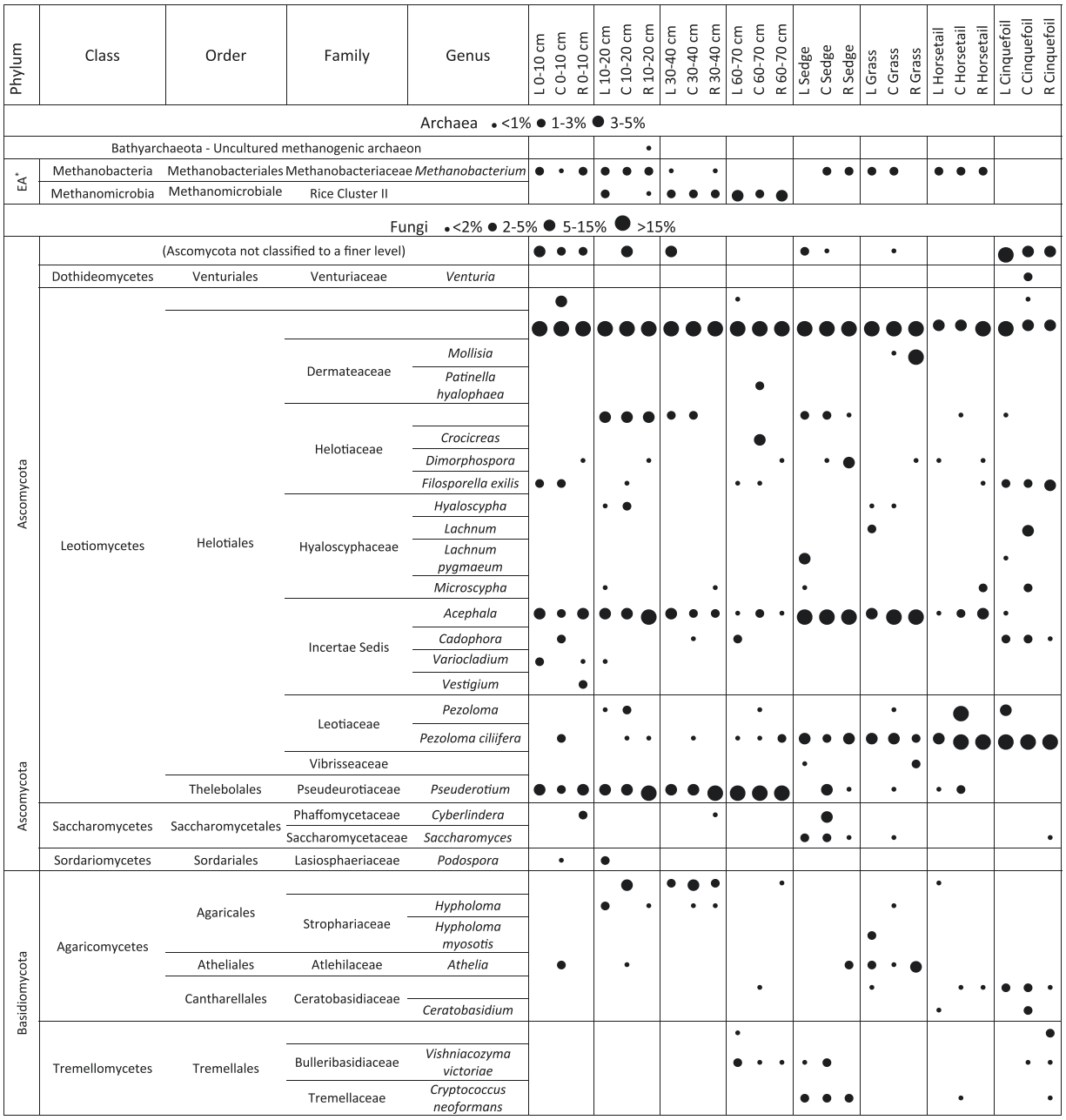

FIG 2 Top summed OTUs of like taxonomic groups that comprise at least 1% (prokaryotes) or 2% (fungi) relative abundance in one or more experimental treatments. This arbitrary cutoff accounts for the top 19 to 24% of prokaryote reads and the top 81 to 96% of fungal reads per bulk peat treatment category and the top 34 to 39% and 81 to 91% of prokaryote and fungal root sample reads, respectively. Relative abundances correspond to the most specific taxa to which they were classified. For example, if several OTUs were identified to order but not finer resolution (family, genus), these were grouped in one line/dot labeled with that order (Helotiales, for example). That line/dot does not include those OTUs that are of the same order but identified to a finer resolution (OTUs of the genus *Acephala* of the order Helotiales, for example, are included on a different line). If an OTU was identified to the genus, everything of that genus appears on the same line/dot but is not included in the line mapped only to the corresponding order. *, phyla names unable to fit in the figure: T, *Tenericutes*; VM, *Verrucomicrobia*; EA, *Euryarchaeota*.

composition was the most distinct between the 10- to 20-cm and the 60- to 70-cm depths. Between these depths, the main differences included the increase in relative abundance of Thelebolales with depth as the Helotiales decreased (Fig. 2). Of the fungi, five taxa were significantly different at the 0- to 10-cm depth between the lowered and raised water table treatments ($P < 0.01$, differential abundance) (Table S2c). These taxa included the order Polyporales of the Basidiomycota, containing saprotrophs able to

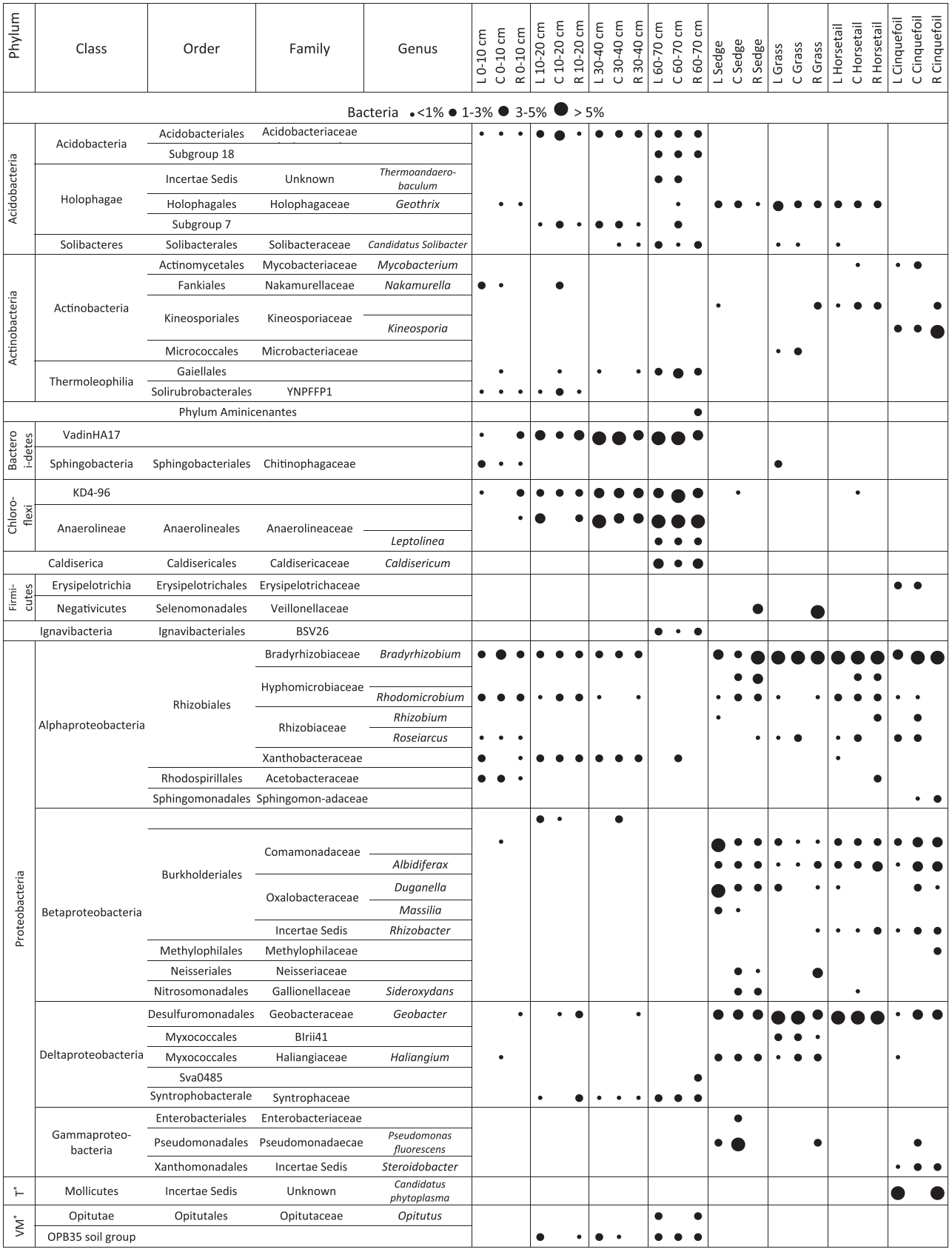

FIG 2 (Continued)

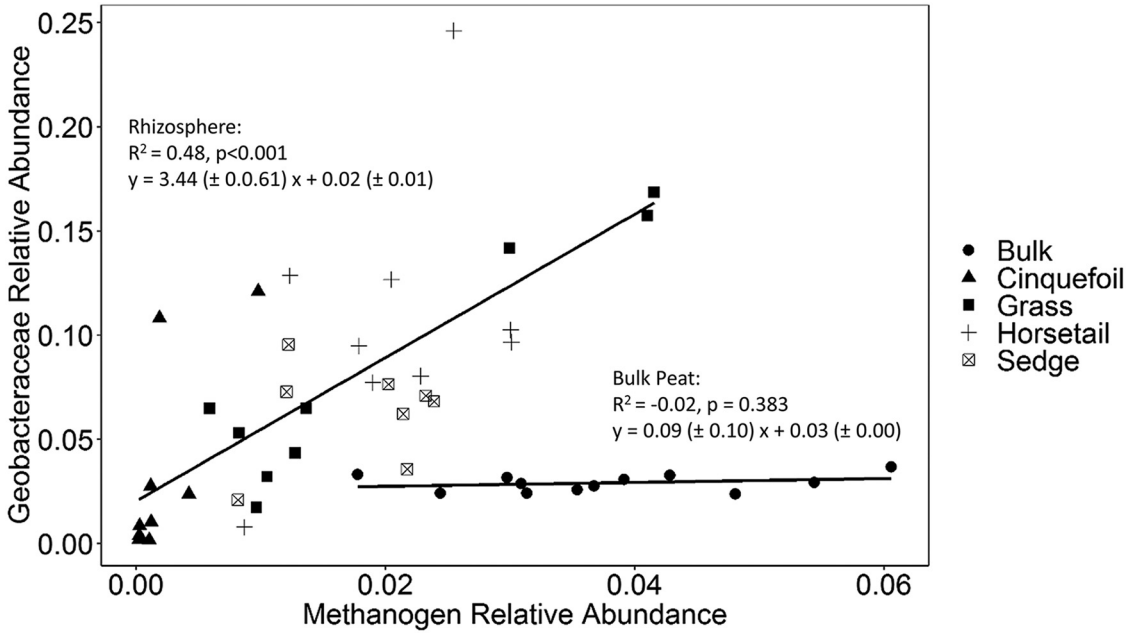

FIG 3 Cooccurrence between relative abundances of methanogens and family *Geobacteraceae* in the rhizosphere versus the lower rooting zone (rhizosphere) bulk peat (10- to 20-cm depth).

degrade lignocellulosic organic matter (white rot fungi), which was enriched in the lowered water table treatment compared with the raised treatment. A similar pattern was seen in other white rot fungi, particularly the genus *Hypholoma*.

Several taxa of key biogeochemical and functional importance, many associated with iron or methane cycling, appeared to have an affinity for specific plant functional groups. The bacterial genera *Geobacter* (organic compound oxidizing/iron reducing) and *Methylomonas* (methanotrophy) and the family *Gallionellaceae* (iron oxidizing, organic compound cycling) had greater relative abundance in the rhizosphere communities of horsetail, sedge, and grass roots than in cinquefoil roots or bulk peat. The iron-reducing genus *Albidiferax* was more abundant in horsetail, cinquefoil, and sedge roots than in grass roots and bulk peat. Of these, *Geobacter* presence correlated with the relative abundance of rhizosphere-dwelling methanogens in the root samples but not in the bulk peat (Fig. 3).

Hydrogenotrophic methanogen taxa dominated over acetrophic methanogens in the fen. One of two main methanogenic genera, *Methanobacterium*, was found around the roots of sedge, grass, and horsetail but not cinquefoil. Cinquefoil roots had a smaller relative abundance of the genus *Methanobacterium* than even the deeper-rooting-zone bulk peat (10 to 20 cm, $P = 0.01$; 0 to 10 cm, $P = 0.42$). The absolute abundance of the class *Methanobacteria* was greatest at the 10- to 20-cm depth interval (average of 376 ± 91 reads at 10 to 20 cm versus 55 ± 37 reads at 60 to 70 cm, control water table treatment). The other most abundant methanogenic taxon, the order *Methanomicrobiales* (Rice Cluster II), had a higher relative abundance, and absolute abundance of its class *Methanomicrobia*, at the 60- to 70-cm depth (Fig. 2 and Fig. 4) (196 ± 95 reads at 10 to 20 cm versus 864 ± 123 reads at the 60- to 70-cm interval, control water table treatment). The greatest average relative abundance of methanogens was found in the raised water table treatment at 10 to 20 cm (Fig. 5).

Methanotroph-containing genera *Methylosinus*, *Methylomonas*, *Roseiarcus* (all *Alphaproteobacteria*) (36, 37), and "*Candidatus* Methylacidiphilum" (*Verrucomicrobia*) (38) were found. *Methylomonas* was enriched in sedge, grass, and horsetail roots ($P < 0.01$) (Table S3).

Dominant taxa in a boreal rich fen. The top 10 prokaryote phyla in the bulk peat were *Acidobacteria*, *Actinobacteria*, *Aminicinantes*, *Bacteroidetes*, *Chloroflexi*, *Euryarchaeota*, *Ignavibacteriae*, *Proteobacteria*, and *Verrucomicrobia* (Fig. S1). The fungal samples in general

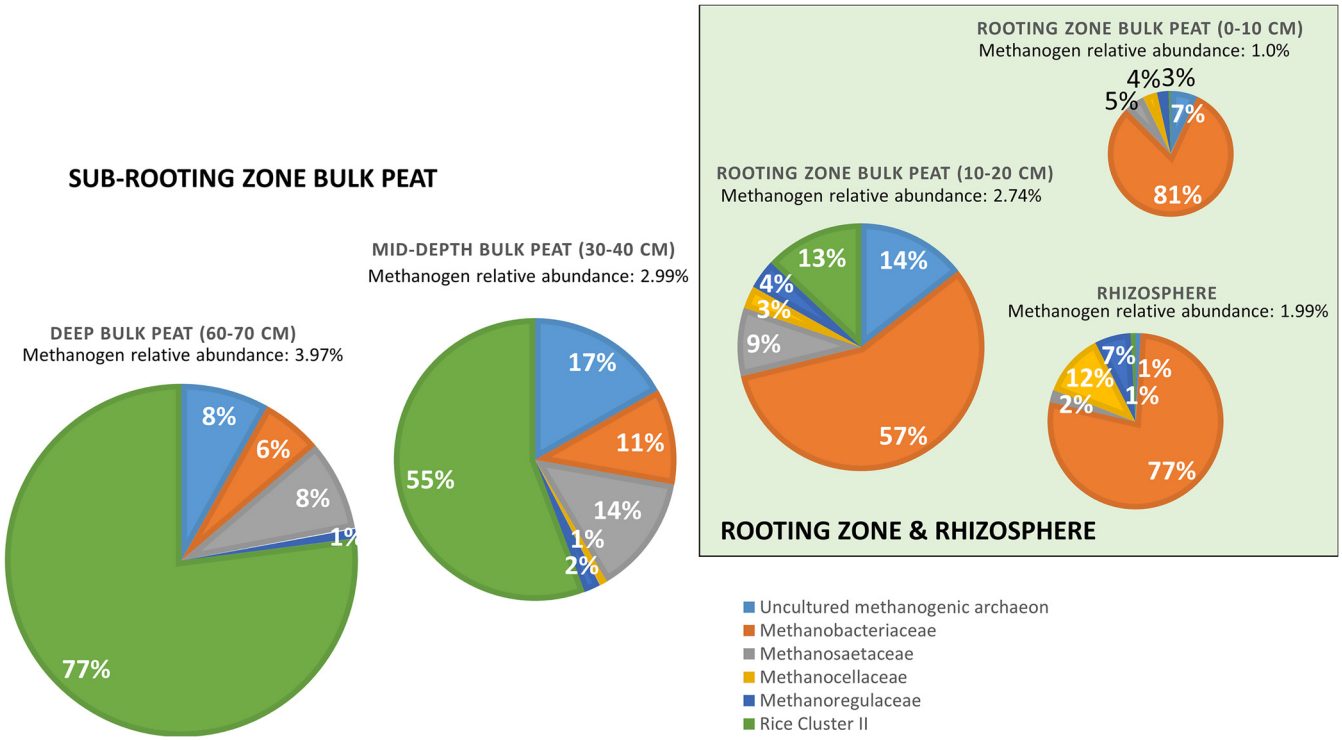

FIG 4 Methanogen community composition by family in bulk peat by depth and the root-associated or rhizosphere community (control water table treatment).

were dominated by the Ascomycota, specifically order Helotiales. Other dominant orders included Thelebolales, Pleosporales, Agaricales, and Tremellales (Fig. 2, Fig. S2).

The top 10 prokaryote phyla in the plant functional group rhizospheres were *Acidobacteria*, *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Euryarchaeota*, *Planctomycetes*, *Proteobacteria*, *Tenericutes*, and *Verrucomicrobia* (Fig. S1, Fig. 2). The rhizosphere fungal samples, as in the bulk peat, were dominated by the Ascomycota order Helotiales. Other dominant orders included Thelebolales, Agaricales, Tremellales, and Cantharellales (in the rhizosphere of cinquefoil) (Fig. S2). Particularly abundant in the root samples were the bacterial genera *Bradyrhizobium* of the *Alphaproteobacteria* and *Geobacter* of the *Deltaproteobacteria*, together often making up >10% of the total fine root reads. *Tenericutes*, which was classified as "*Candidatus* phytoplasma," or Brinjal little leaf phytoplasma—a possible bacterial pathogen—dominated several of the cinquefoil samples. The genus *Acephala* and species *Pezoloma ciliifera* were particularly abundant in the root samples in general. Possible fungal pathogens/saprobes *Filosporella exilis*, family Tremellaceae, and family Ceratobasidiaceae (39) were also enriched in cinquefoil roots. In the lab, attempts were made to sterilize the root surface on several specimens to distinguish microorganisms (specifically fungi) living inside the roots from those associated with the root surface. The one successfully sequenced surface-sterilized grass root sample was dominated by *Acephala* and *Mollisia* spp., together accounting for 61% of reads. Arbuscular mycorrhizal fungi (AMF), mainly associated with the grass and cinquefoil roots, were dominated by Archaeosporales of the phylum Glomeromycota. Reads of the AMF genus *Glomus* (40) were also present in cinquefoil roots. AMF were unaffected by water table treatment in both the cinquefoil and grass roots.

DISCUSSION

This study demonstrated that water table treatment and plant functional groups structure the microbial community in a subarctic rich fen. Additionally, the results

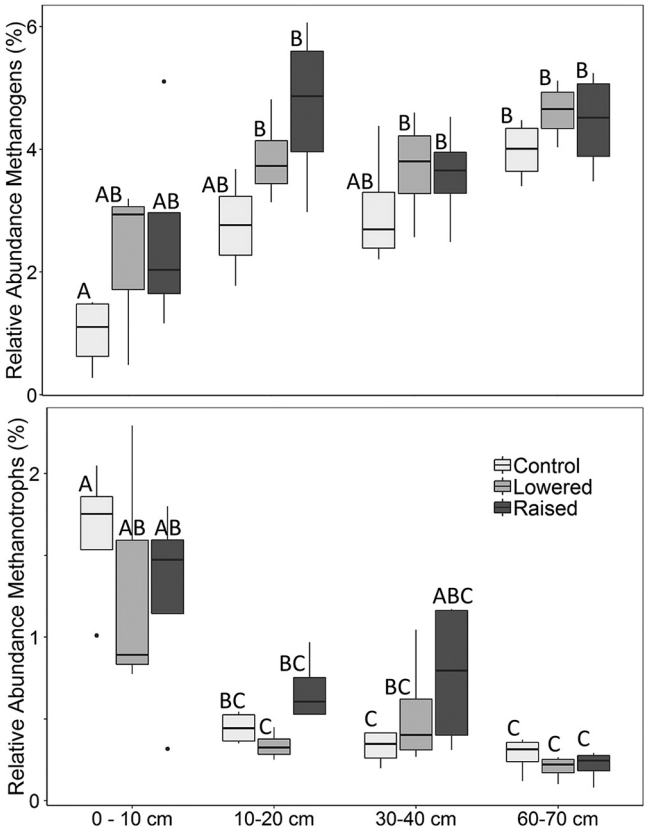

FIG 5 Relative abundances (%) of methanogens and methanotrophs in the water table treatments for bulk peat ($n = 4$ peat cores). The box plot shows the 25th and 75th percentiles and 95% confidence interval, with letters indicating pairwise differences ($P < 0.05$).

provided insights into the community composition and microbial interactions related to carbon cycling in this ecosystem.

The effects of the water table treatments are most evident in the lower rooting zone (10- to 20-cm interval) of the bulk peat. This zone is likely most directly affected by hydrologic shifts, as has been suggested by others (41) and is represented in an overview with associated plant functional groups and major microbial community components of interest (Fig. 6). Here, there is an apparent relationship between plant functional group rhizospheres, bacterial communities associated with both iron cycling and methanogenesis, and possible fungal endophytes—all of which appear to react to water table changes.

Rhizosphere iron reducers and methanogens appear to be occupying similar ecological niches around the plant roots in this fen (Fig. 3). The microorganisms likely associated with iron cycling made up anywhere from roughly 1 to 20% of the amplicons from plant functional group roots, compared with 2% or less of the reads associated with bulk peat. The study fen is iron rich, supporting iron-mediated biogeochemical processes (42). *Geobacter* has been associated with the rhizospheres of rice plants and other freshwater and marine plants, due to the consumption of root exudates. Aerenchymatous roots also provide an environment with fluctuating redox potential via oxygen delivery to the rhizosphere, which is helpful for continual iron oxidation and reduction by microorganisms (43, 44). The 10- to 20-cm zone also boasts the greatest relative abundance of the methanogenic *Methanobacteria*, which were additionally enriched around the roots of sedges, grasses, and horsetail. The greatest average relative abundance of all methanogens was found in the raised water table treatment at 10 to 20 cm, consistent with long-term methane (CH_4) efflux data at this experimental site, which showed the highest efflux rates from the raised water table treatment (45).

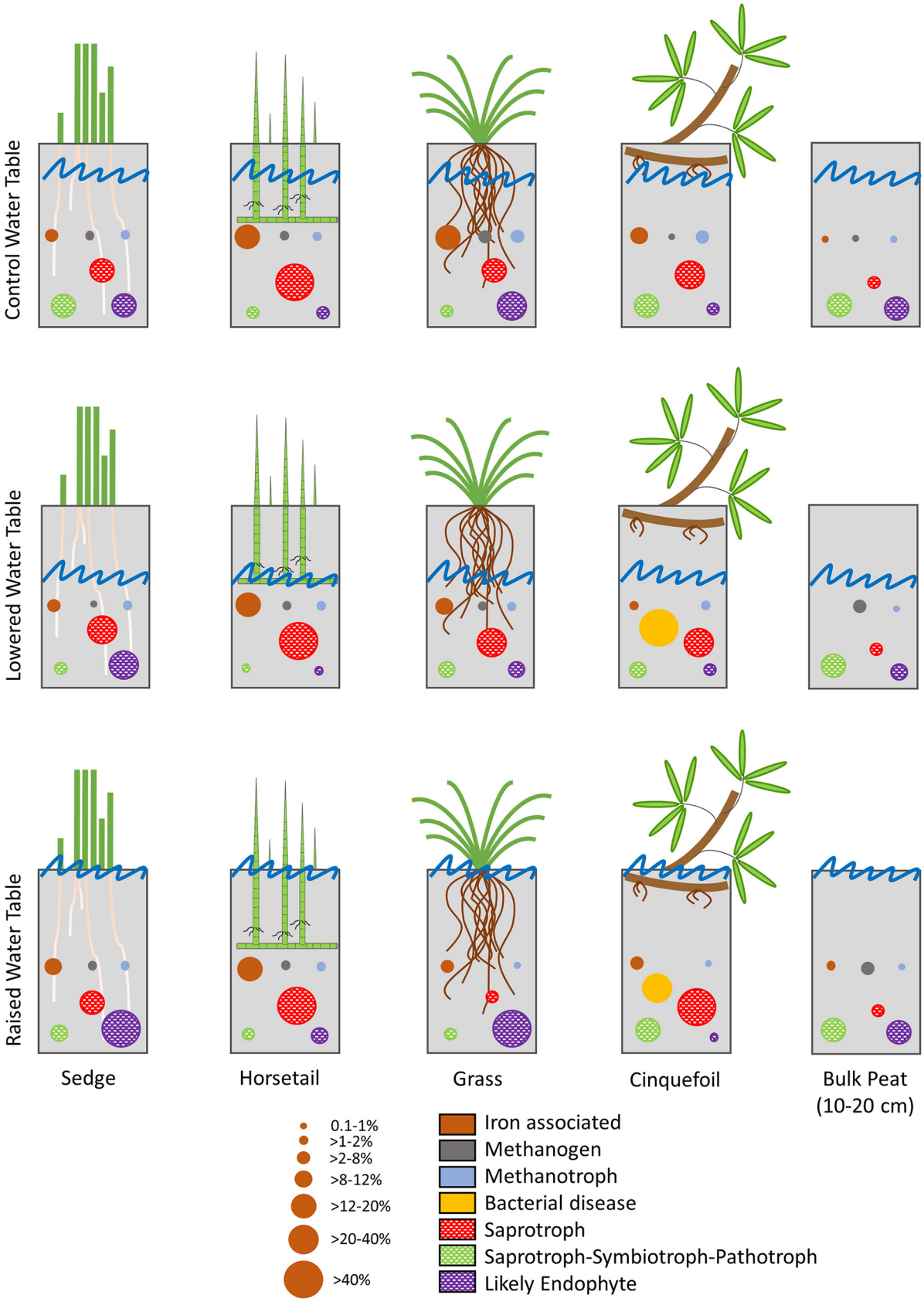

FIG 6 Generalized summary of findings in the rooting zone and lower rhizosphere by relative abundance (functional groupings of the top 20 OTUs plus taxa of statistical interest mentioned in Results). The top row represents the control, the middle row represents the lowered water table treatment, and

(Continued on next page)

Stimulation of methanogenesis by root exudates, including those from sedges, is substantial in fens (46–48). It is possible that both methanogens and iron cycling organisms cooccur in the niche environment associated with roots due to the specific conditions there; it is unknown if these two groups interact with each other in this environment. Previous research suggests that methanogens sometimes use Fe(III) as an electron acceptor (49) but also demonstrates the ability to develop symbiotic relationships with iron reducers, using semiconductive iron to transfer electrons between organisms (50–52). Direct interspecies electron transfer has recently been identified as a syntrophic metabolism between *Geobacter metallireducens* and a species of *Methanobacterium* (53), both families of which are enriched around roots in this experiment (Fig. 3 and 4). Additionally, iron reducers have been documented to use CH_4-derived carbon in sub-Arctic Alaskan lakes (54). Further study would be needed to explore if syntrophic or other complex relationships between iron cyclers and methanogens, facilitated by the plant rhizosphere environment, occur in this iron-rich fen.

Interestingly, there were two distinct groups of archaeal methanogens, one "deep peat" community (class *Methanomicrobia*), of greatest amplicon abundance at the 60- to 70-cm depth and one previously mentioned "rhizosphere" community (class *Methanobacteria*). Both of these classes of archaea are among the dominant methanogens found across a wide range of Alaskan wetlands (55). The stark difference in habitat preference could allude to differences in obligate living conditions, metabolic pathways, or capability of or preference for biological relationships. For example, syntrophic hydrogenotrophic methanogenesis involving propionate reduction around roots occurs in rice paddies, demonstrating that the rhizosphere provides energetically favorable opportunities for some methanogens (34, 56).

Another partnership of possible importance in this rich fen is between fungal root endophytes and graminoids (sedges and grasses), which both decline in relative abundance with a lowered water table (6). *Mollisia*, *Acephala*, and *Mortierella* were significantly enriched in the sedge, grass, and surface-sterilized grass root samples. *Mollisia* has previously been reported to be associated with grass roots (57). All three of these genera have also been documented to act as root endophytes (58–60). Some endophytes have the ability to foster disease resistance for their hosts, including members of the *Mortierella* (61). Furthermore, *Acephala* spp. isolated from peat—with 98% similarity to one of the dominant OTUs in this study (including surface-sterilized root samples)—have been shown capable of Fenton chemistry/quinone redox cycling as a biodegradation tool. Fenton chemistry is a biochemical process which utilizes iron cycling to ultimately create powerful oxidants and carbon dioxide (CO_2) (62). Given the apparent biological importance of iron cycling in this fen, there could hypothetically be both chemical and biological resources (redox-active environment, high concentration of iron, and bacteria actively transforming/cycling iron) available to support organisms performing these biochemical pathways. Further research would be needed to understand whether fungal endophytes performing Fenton chemistry/quinone redox cycling are present. Understanding the effect of this pathway could significantly improve our understanding of decomposition dynamics and net CO_2 emissions at this rich fen. Additional effects of water table treatment on fungal decomposition include enriched lignin-degrading (white rot) fungi in the lowered water table treatment. A fungal-mediated increase in the capacity for lignin oxidation could suggest additional pathways for carbon

FIG 6 Legend (Continued)
the bottom row represents the raised water table treatment, which is true to the spatial placement of the treatments. The plant representations from left to right are as follows: sedges, horsetail, grasses, cinquefoil, and bulk peat (10 to 20 cm). Represented genera for iron associates are *Geobacter* (*Geobacteraceae*), *Albidiferax* (*Comamonadaceae*), *Sideroxydans* (*Gallionellaceae*), *Geothrix* (*Holophagaceae*), and *Ferribacterium* (*Rhodocyclaceae*); for methanogens are *Methanobacterium*; and for methanotrophs are *Methylomonas*, *Methylosinus*, *Roseiarcus*, and "*Candidatus* Methylacidiphilum." Bacterial disease, Brinjal little leaf phytoplasma (*Tenericutes*). Fungal functional groups (saprotroph, saprotroph-symbiotroph-pathotroph, likely endophyte) are derived from literature mentioned in the text and assignments by FUNGuild. All binned relative abundance (rel. ab.) categories are true to each replicate ± 5% of the value (rel. ab. % · 0.05; $n = 3$ for plant roots, $n = 4$ for peat cores) except for the bacterial disease category in cinquefoil, which either occupied >70% of total reads (2 of 3 replicates in lowered water table treatment and 1 of 3 in raised water table treatment) or <0.01%.

loss with a lowered water table (63), though interactive effects with changes in vegetation community would also have to be explored to verify this in the field (64, 65).

Finally, the microbial community around the marsh cinquefoil rhizosphere may be inhospitable to certain bacterial and archaeal taxa—inferred from the low relative abundances of certain taxa found there. This could be due to a lack of attractive root exudates, a lack of aerenchyma or gas transport to the rhizosphere, a shallower rooting zone, antagonistic biochemical outputs, or disease. This observation is supported by previous work demonstrating slower decomposition rates in cinquefoil-only mesocosms in comparison to the same plant functional groups (grass, horsetail, sedge) (19). Cinquefoil was, in this study, likely under pressure from bacterial and possibly fungal disease, which could skew read relative abundance. Plant parasites and diseases in northern latitudes are expected to intensify due to climate and associated landscape change (66, 67); it could be possible that we observed a simulated (by long-term experiment) or actual (climate change) response to a shifting environment (68).

Conclusion. Taken together, these findings suggest that the rhizosphere is arguably the most important and dynamic biogeochemical zone in carbon and iron cycling, which appear to be linked in this rich fen ecosystem. Hydrology and the water table do play an important role, governing oxic versus anoxic environments in peatlands, but also governing which species of plants live in an ecosystem (6). The number of OTUs significantly affected by the water table treatments was greatest at the 10- to 20-cm depth, which coincides with the deep rhizosphere. These organisms included the white rot fungi and lignocellulose degraders, suggesting a higher rate of fungal decomposition in the lowered water table treatment. Conversely, fungal endophytes were associated with grass and sedge roots and were enriched in the raised water table treatment. Iron-cycling bacteria and methanogens of the *Methanobacteria* were enriched around the roots of horsetail, sedges, and grasses. These taxa may gain greater biogeochemical influence, as projected higher precipitation rates could lead to an increased abundance of sedges and grasses in boreal fens (see also reference 69). A distinct, deep peat-dwelling methanogen population, largely of the *Methanomicrobia*, was most enriched at the 60- to 70-cm peat depth. Future research should examine (i) specific rhizosphere interactions of iron reducers and methanogens in iron-rich fens and (ii) in-depth interactions of root endophytes with iron and carbon cycling, with a focus on greenhouse gas production.

MATERIALS AND METHODS

Field site. The Alaska Peatland Experiment (APEX) rich fen (pH 5.6 to 5.9) is located southwest of Fairbanks, Alaska, in the floodplain of the Tanana River. APEX is a long-term wetland monitoring and research project examining how climate change will affect carbon cycling via water table and plant community shifts (45). As such, experimental water table manipulation treatments (lowered, raised, and control) have been in place since the initiation of the project in 2005 and have been maintained every year since, except for years in which the fen has flooded. The fen is a dynamic ecosystem, experiencing years in which the water table remains below the surface of the peat and years of heavy flooding, with the water table persisting up to a meter above the peat surface. Since the initiation of the project in 2005, the fen has experienced heavy flooding during 5 seasons (2008, 2014, post-July 2016, 2017, and 2018) (68). During the time of sample collection (June 2016), the experimental water table treatments had been maintained for an entire growing season the year prior (2015) and the first half of the growing season of 2016. For context, the order of the water table treatments in space is control, lowered, and raised, separated by approximately 50 m in between each. Therefore, some of the differences in the results (i.e., differences between control and raised) may be influenced more by spatial location/distance than by water table treatment if little variation is seen between the lowered versus raised treatments, which are located next to each other. The site is rich in calcium (~14 mg/liter pore water) (70) and iron (Fe), concentrations of which are dominated by organically bound Fe (2,700 to 6,200 mg/kg peat) (42). Previous work has described in detail changes in soil properties (42) and vegetation (6, 35) and their resultant effects on biogeochemical processes and trace gas effluxes in response to the water table treatment effects over time (45, 70–72). Dominant plant species include *Carex atherodes* Spreng. (a sedge), *Calamagrostis* sp. Michx (a grass), *Equisetum fluviatile* L. (a horsetail), and *Comarum palustre* L. (marsh cinquefoil) (6) and were targets of the plant functional group analysis.

Peat core collection. In June of 2016, four peat cores were taken from each of the three water table treatment plots using a sharpened 6-cm-diameter stainless steel corer fitted with an adapter to a power drill (73). In the field, cores were placed on a fresh sheet of aluminum foil for further processing. Each core was subsampled at four depth intervals—1 to 10 cm, 10 to 20 cm, 30 to 40 cm, and 60 to 70 cm. Nitrile gloves were replaced with fresh gloves between cores, and the distinct depth segments were

only touched using the inside of a prelabeled Whirl-Pak bag. Samples were immediately transferred to a cooler and stored on ice until transportation to the lab on the same day. All samples were immediately frozen at −20°C and shipped frozen to the Northern Research Station in Houghton, MI, where they were stored at −20°C until laboratory processing.

Fine root collection. To test for differences among plant functional groups, three replicates of each dominant plant species (horsetail, sedge, cinquefoil, and grass; see above) were collected from each of the three water table treatment plots. To avoid disturbance of the long-term experimental plots, plants were collected outside the main long-term study area, where the cores were taken, but still in the area influenced by the water table treatments. Collection was performed as follows: a 10-cm by 10-cm square was cut around the desired plant to a depth of 20 cm. The resulting volume of peat was removed from the wetland with gloved hands, and the peat was removed as best as possible from the roots. No rinsing was performed in the field. The above-ground portion of the plant was severed, and the remaining root system was placed in a Whirl-Pak bag and then in a cooler for transport back to the lab. Roots were refrigerated and then shipped on ice to the Northern Research Station in Houghton, MI, where they were stored at −20°C until laboratory processing. In the lab, each frozen root sample was removed from the freezer and placed between freezer packs in an insulated box. Using flame-sterilized scissors and gloved hands, sections of the fine root system were snipped from the root mass and placed immediately in 95% ethanol in a petri dish. Fine roots were manually cleaned of peat using a dissecting microscope and flame-sterilized forceps and sorted into a second "clean roots" petri dish with 95% ethanol. Only fine roots that had been living at the time of collection were selected for further processing, based on color and turgor.

Molecular methods for sampling bacteria, archaea, and fungi. (i) Peat cores. Approximately 10 ml of peat from a sample was placed in a 50-ml tube, followed by 20 3.2-mm chrome-steel beads, and pulverized with a modified Mini-BeadBeater-96 (BioSpec Products, Bartlesville, OK, USA) for 2 min. DNA was then extracted from a 0.5-g subsample of the pulverized peat using a PowerSoil DNA (MoBio Laboratories, Inc., Carlsbad, CA, USA) isolation kit following the manufacturer's instructions, with the inclusion of a 30-min incubation at 65°C following the addition of the C1 lysis buffer and 10 min of vortexing. DNA was cleaned with a MoBio PowerClean Pro DNA cleanup kit and quantified with a Qubit fluorometer (Invitrogen, Life Technologies, Carlsbad, CA, USA). DNA was then subjected to a test PCR to ensure that it could be amplified, and products from the PCR were examined on an agarose gel.

(ii) Fine roots. Depending on the species and size of the root system, three to nine 2- to 3-cm lengths of fine root from different parts of the fine root system were isolated into 2-ml centrifuge tubes with 0.5 ml 95% ethanol. An additional small subset of sedge and grass roots was surface sterilized using 30% H_2O_2 for 1 min and then rinsed thrice with Nanopure autoclaved water and transferred to 0.5 ml 95% ethanol.

Ethanol was evaporated from the samples using a CentriVap and then immediately freeze-dried for 72 h. After drying, 10 3.2-mm chrome-steel beads (BioSpec Products, Bartlesville, OK, USA) were added to each tube, and samples were subjected to bead beating for 45 s. DNA was extracted and purified from the pulverized roots using the Qiagen DNeasy plant minikit and MoBio PowerClean DNA cleanup kits, respectively, following the manufacturer's protocol. The following methods were the same as for those of the peat cores, described above.

(iii) Sequencing. Community amplicon sequencing was conducted at the U.S. Department of Energy Joint Genome Institute (JGI, Walnut Creek, CA, USA). Sample and library prep were performed according to the methods of Caporaso et al. (99), Tremblay et al. (74), Coleman-Derr (75), and Lamit et al. (29). The fungal internal transcribed spacer 2 (ITS2) region was targeted using the forward primer fITS9 (76) and the reverse primer ITS4 (77), and the V4 region of the prokaryote (bacteria and archaea) 16S DNA gene was targeted using the forward primer 515f and reverse primer 806r (78). A peptide nucleic acid (PNA) clamp was used to exclude plastids and mitochondria in the root samples (79). Primers were fitted with Illumina sequencing adaptors, and the reverse primer contained an 11-bp index unique to each sample. Samples were pooled into equimolar aliquots and sequenced on an Illumina MiSeq platform (Illumina, Inc., San Diego, CA) using 2 × 300-bp chemistry. Data are available through the JGI genome portal (project IDs 1141768 and 1127271, http://genome.jgi.doe.gov/).

Bioinformatic processing. Bioinformatic processing of the resulting microbial sequence data was conducted with the JGI BBtools suite and the Quantitative Insights into Microbial Ecology pipeline (QIIME1) (80). BBMap 37.58 (https://sourceforge.net/projects/bbmap/) was used to perform adapter trimming and to filter PhiX 174 from raw interleaved fastq files using bbduk.sh. Primers were trimmed using Cutadapt 1.14 (81). Primer-trimmed sequences were merged using BBMerge (bbduk.sh, minimum overlap = 30 bp, max error rate 0.3). Merged sequences were quality-filtered in QIIME 1.9.1 with VSEARCH 2.4.2 using a minimum bp length of 100, maximum expected errors of 0.5, and maximum number of Ns (i.e., ambiguous bases) of 0. After barcode extraction, demultiplexing was completed with a quality parameter set, so there was no filtering at this step.

V4 region of 16S rRNA genes (bacteria and archaea). Reference-based chimera detection was completed with USEARCH61 (82) trained to the SILVA 128 (83) database at 97% sequence similarity. Open reference OTU picking was performed using the UCLUST (82) clustering tool at 97% sequence similarity. Representative sequences were assigned taxonomy with the Ribosomal Database Project (RDP) Classifier 2.2 (84) with confidence set at 0.8 (85) trained against the SILVA 128 QIIME release reference data set. Mitochondria, chloroplasts, unclassified sequences, and underrepresented sequences (<0.005% across the entire data set) were filtered from the final OTU table. The final OTU table was rarefied to 25,037 sequences per sample, the size of the smallest sample, using the phyloseq package in R 3.5.1 (86), where the

rest of the analyses were completed. Functional groups for prokaryotes were assigned to the most abundant taxa via literature review.

ITS2 (fungi). The ITS region of the sequences was extracted using ITSx 1.1 (87). Additional nonfungal sequences were identified using a closed-reference OTU picking method at 100% similarity against a hand-curated NCBI data set of nonfungal ITS2 adapted (script and data set available at https://github.com/gzahn/Format_NCBI_QIIME) and filtered from the data set. OTU clustering was completed using an open reference approach (88) using UCLUST with 97% sequence similarity (89). Taxonomy was assigned using the RDP Classifier trained with the UNITE 7.2 species hypothesis dynamic clustering data set (released 1 December 2017). Any additional OTUs classified as nonfungal and unidentified were filtered from the data set, and sequences classified only to a fungal phylum were put through BLASTn searches in the NCBI nucleotide database. If tested OTUs were clearly of fungal origin with an E value of $\leq 1 \times 10^{-20}$, they were retained. Low-occurrence OTUs (<10 reads) were filtered from the data set, and tentative functional group assignment was completed using FUNGuild (90). The final OTU table was rarefied to 4,098 sequences per sample, the size of the smallest sample, in R using the phyloseq 1.24.2 package (91). Percentage similarities between sequences in this study and published or documented species hypotheses of interest to further elucidate functionality were aligned and compared with the MUSCLE (92) plugin in UGENE 1.32 (93).

Statistical analysis. Statistical analysis was completed in R statistical software (86) and PRIMER (94). Statistical significance for all tests was accepted at $P < 0.05$. Reports and comparisons regarding overall community abundance are reported as relative abundance values. The DESeq 1.34.1 package (95) was used to calculate differential abundances of OTUs between treatments, with threshold cutoffs of $\log_2$ fold change of >2 and adjusted $P < 0.01$. A $\log_2$ fold change of 1 indicates that an OTU is two times as abundant in one treatment compared to another. For example, if treatment A contains 50 reads of OTU A and treatment B contains 25 reads, the $\log_2$ fold change would be $\log_2 (50/25) = 1$, i.e., one 2-fold change. Conversely, if treatment A contained 10 reads and treatment B contained 20, the $\log_2$ fold change would be $\log_2 (10/20) = -1$. One-way analysis of variance (aov function) was used to compare specific microbial taxa between treatments at specific depths, using Tukey's honest significance difference *post hoc* test. Canonical analysis of principal coordinates (CAP) was performed with the Bray-Curtis dissimilarity metric in phyloseq to visualize differences in community structure and composition. For bulk peat, PERMANOVA models included water table treatment, peat depth, and their two-way interaction as fixed effects, with core as a random effect. For rhizosphere communities, PERMANOVA included water table treatment, plant species, and their two-way interaction. These more complex PERMANOVA analyses were run with Bray-Curtis dissimilarity in Primer 6.1.15 with PERMANOVA+ 1.0.5 (PRIMER-E, Plymouth, UK) (96), using type III sums of squares and *P* values obtained by permuting reduced models lacking the specific factor being tested (96). Between-taxa correlations and *P* values at the same phylogenetic level (i.e., genus, class, family) were obtained from a correlation matrix created using the package Hmisc 4.2-0 (97).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.8 MB.

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We declare that we have no conflict of interest.

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