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ARTICLE

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Experimental erosion of microbial diversity decreases soil CH_4 consumption rates

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Abstract

Biodiversity-ecosystem functioning (BEF) experiments have predominantly focused on communities of higher organisms, in particular plants, with comparably little known to date about the relevance of biodiversity for microbially driven biogeochemical processes. Methanotrophic bacteria play a key role in Earth's methane (CH_4) cycle by removing atmospheric CH_4 and reducing emissions from methanogenesis in wetlands and landfills. Here, we used a dilution-to-extinction approach to simulate diversity loss in a methanotrophic landfill cover soil community. Replicate samples were diluted 10^1–10^7-fold, preincubated under a high CH_4 atmosphere for microbial communities to recover to comparable size, and then incubated for 86 days at constant or diurnally cycling temperature. We hypothesize that (1) CH_4 consumption decreases as methanotrophic diversity is lost, and (2) this effect is more pronounced under variable temperatures. Net CH_4 consumption was determined by gas chromatography. Microbial community composition was determined by DNA extraction and sequencing of amplicons specific to methanotrophs and bacteria (*pmoA* and 16S gene fragments). The richness of operational taxonomic units (OTU) of methanotrophic and nonmethanotrophic bacteria decreased approximately linearly with *log*-dilution. CH_4 consumption decreased with the number of OTUs lost, independent of community size. These effects were independent of temperature cycling. The diversity effects we found occured in relatively diverse communities, challenging the notion of high functional redundancy mediating high resistance to diversity erosion in natural microbial systems. The effects also resemble the ones for higher organisms, suggesting that BEF relationships are universal across taxa and spatial scales.

KEYWORDS
biodiversity effects, DNA analysis, greenhouse gas, methane, soil microbes

INTRODUCTION

Over the past decades, experimental (Cardinale et al., 2011), observational (e.g., Flombaum & Sala, 2008; Liang et al., 2007; Oehri et al., 2017), and theoretical studies (e.g., de Mazancourt et al., 2013) have demonstrated that biodiversity is critical for the provisioning of a wide range of ecosystem services. Most biodiversity experiments to date have focused on plant-related functions such as primary productivity. In general, productivity was found to increase with the diversity of species present, although the additional benefit mediated by extra species diminishes when communities already contain many species.

Plants have evolved a fascinating diversity of organisms adapted to a wide range of environments. Nevertheless, they are metabolically relatively uniform and drive essentially the same suite of biogeochemical processes. In contrast, soil microbes drive a plethora of chemically extremely diversified ecosystem functions. The ecology and life history of microbes differ from the ones of plants and animals, potentially giving rise to divergent mechanisms underpinning microbial biodiversity-ecosystem functioning (BEF) relationships (Krause et al., 2014). The diversity of microorganisms in soils is also extreme, exceeding the one found in higher organisms by orders of magnitude (Locey & Lennon, 2016; Roesch et al., 2007; Sogin et al., 2006; Torsvik et al., 1990). This supports the idea that microbial communities are less susceptible to diversity loss due to high functional redundancy. Conversely, soils are extremely heterogeneous at the microscale, and some key microbial functions are carried out by phylogenetically relatively narrow groups of microbes. One example is soil methanotrophic bacteria. Methanotrophs constitute the only relevant biogenic sink for atmospheric methane (CH_4), an important anthropogenic greenhouse gas (IPCC, 2021). In particular in wetlands and landfills, methanotrophs also remove CH_4 produced in the soil by methanogenic archaea, and functionally thus act as a "biofilter" that eliminates a large fraction of potential soil CH_4 emissions (Conrad, 2009). Methanotrophic bacteria belong to either the Gammaproteobacteria (type I methanotrophs), Alphaproteobacteria (type II methanotrophs), Verrucomicrobia (type III methanotrophs; Knief, 2015; McDonald et al., 2008), or to a newly discovered methanotrophic type of Actinobacteria (van Spanning et al., 2022). In typical habitats, their diversity rarely exceeds a few dozen strains. It may thus well be that CH_4 oxidation rates by these communities critically depend on the number of species present. However, experimental microbial BEF research has only recently begun to emerge (Bell et al., 2005; Bier et al., 2015; Delgado-Baquerizo et al., 2016; Fetzer et al., 2015; Ho et al., 2014; Replansky & Bell, 2009; Schnyder et al., 2018; Wertz et al., 2006, 2007). Therefore, to date, little is understood on how microbe-mediated ecosystem functions depend on the diversity of the respective guilds.

Here, we analyze diversity effects on CH_4 consumption in microbial communities extracted from the cover soil of a landfill that had accumulated several million cubic meters of municipal solid waste until the 1990s. In such cover soils, methanotrophs oxidize CH_4 at high rates, thereby filtering most CH_4 produced in the course of waste degradation (Henneberger et al., 2012; Schroth et al., 2012). Nevertheless, landfills remain a major global anthropogenic source of CH_4, and any temporary or permanent reduction in methanotrophic activity in cover soils can trigger massive increases in net CH_4 emissions. To test whether methanotrophic activity in such communities is impaired by diversity loss, we used a dilution-to-extinction approach to create model communities spanning a gradient in the diversity of taxonomic units. This dilution method allows working with microbial members difficult to cultivate, and the resulting diversity gradients mimic a realistic extinction scenario in which rare species and species that recover slowly from disturbance are more likely to become extinct (Giller et al., 2004). We created replicate dilution series ranging from 10^{-1} to 10^{-7}, inoculated microcosms with these dilutions, and preincubated them for 1 month to equalize community sizes. Then, microcosms were incubated for 3 months under an atmosphere with an average of 7400 μmol CH_4 mol^{-1}, either at a constant temperature of 15°C, or at a temperature diurnally cycling between 10 and 20°C. Throughout the experiment, we quantified methanotrophic activity by assessing CH_4 consumption and CO_2 production, the latter including respiration by nonmethanotrophs. Using quantitative PCR (qPCR) of a functional gene that encodes for a subunit of the particulate methane mono-oxygenase enzyme found in most methanotrophs (*pmoA*), we determined methanotrophic community size and growth. Total bacterial abundance was determined as ribosomal gene abundance (16S rRNA subunit). Finally, we determined the composition of the experimental communities by high-throughput amplicon sequencing of ribosomal (16S rRNA) and functional (*pmoA*) gene fragments.

Our aim was to test whether diversity loss affects methanotroph community functioning. We were further interested in whether phylogenetic diversity (PD) is a good predictor of ecosystem functioning. Ultimately, BEF relationships arise from differences in functional traits among species, and PD might serve as a proxy for functional differences if the relevant traits are preserved through evolutionary time (Cadotte et al., 2008). Finally, we asked whether diversity effects are larger in a fluctuating environment (diurnal temperature cycle) that might provide a larger environmental niche space and allow for additional functional complementarity among community members.

MATERIALS AND METHODS

Soil sampling

We collected soil from a landfill (Liestal, Basel-Land, Switzerland, 570 m above sea level [asl] 47°29′ N, 7°45′ E, Gomez et al., 2009) that had accumulated ~3×10^6 m^3 of household, office and construction waste before it was covered with 2 m of soil in the 1990s. The cover soil is a heterogeneous mixture, loamy in texture (40%–60% silt), slightly alkaline (pH 7.3–7.9), and contains pebbles, rocks, boulders and construction material. Annual temperature and precipitation at the site average ~9°C and 1000 mm. We collected soil from a depth of 30–50 cm (~150 cm west of sampling location C1 described in Henneberger et al., 2012), sieved the samples (2 mm mesh), and stored them at 4°C. Before the preparation of the serial dilutions (see below), the soil was incubated for 2 weeks at room temperature under an atmosphere of air spiked with 1% CH_4 (v/v) to re-activate the methanotrophs.

Experimental setup

Inocula were prepared by homogenizing 10 g of the collected landfill soil in 20 ml of sterile H_2O with a pestle and a mortar. This slurry was then repeatedly diluted 10-fold by mixing 1 ml of the highest dilution with 9 ml of sterile H_2O until a final dilution of 10^{-10} was obtained. This procedure was repeated three times with three separate original soil subsamples, resulting in three independent dilution series. From each dilution, 9 ml were transferred to an Erlenmeyer flask with 1 ml NMS (Dedysh et al., 1998) and 40 g γ-sterilized soil (collected at Nenzlingen, Switzerland; 47°33′ N, 7°34′ E, 520 m asl; silty clay loam soil, 41% clay, 52% silt 3.9% C, 0.33% N, pH ≈ 7,8; Niklaus et al., 2003). This matrix soil has very low natural methanotroph abundances, which minimizes interference from remaining soil DNA (the relative abundance of the *pmoA* gene measured by qPCR in this soil was less than 0.01% of that measured later in inoculated microcosms). The flasks were closed with gas-permeable cotton stoppers and incubated in air-tight jars for 1 month to allow methanotrophic communities to recover. During the preincubation, headspace CH_4 concentrations were brought to 1% by injecting CH_4 with a syringe. The flasks were ventilated regularly. Headspace CH_4 was monitored by gas chromatography and additional CH_4 was injected when needed. For the experiment, we used a total of five dilutions: 10^{-1}, 10^{-3}, 10^{-4}, and 10^{-5} showed a recovery of methanotrophic activity during the preincubation, and 10^{-7}, which did not show any significant methanotrophic activity at the end of the preincubation but, as it turned out, recovered during the main experiment. The 10^{-2} dilution had recovered but was excluded to limit the size of the experiment. The experimental microcosms were created by transferring 2 g of cultured soil with an additional 2 g of sterile soil matrix to 50-ml centrifugation tubes (Sarstedt, Nümbrecht, Germany).

Experimental conditions

We incubated each of the 15 distinct communities together with negative controls (sterile soil only) in separate incubators with temperature averages of 15°C (3 dilution series × 5 dilutions × 7 replicates + 2 controls = 107 microcosms per incubator). In the first incubator, the temperature was kept constant. In the second, the temperature ramped up from 10 to 20°C in 12 h, and then down again to 10°C in another 12 h. Within the incubators, the microcosms were kept in air-tight chambers that were connected to a custom-built system that measured and controlled CH_4 concentrations. Headspace air was pumped through a heat exchanger that equilibrated the gas to room temperature. Then, the sampled air was dried in a tube filled with silica gel beads before CH_4 concentrations were determined in a CH_4 detector cell (TGS 2611, Figaro Inc., Arlington Heights, IL). Whenever CH_4 concentrations fell below ~6000 ppm, a mixture of 5% CH_4 and 10% O_2 in N_2 was added through a solenoid valve. The TGS 2611 semiconductor gas sensor provides only approximate concentration measurements, and we therefore used the same sensor and electronics to control CH_4 concentrations in both incubators (switched between incubators with a solenoid valve). The concentration therefore did not differ systematically between the treatments. CH_4 concentrations were further monitored by taking headspace samples from the boxes with a syringe 1–2 times a day. CH_4 concentrations averaged 7400 μmol CH_4 mol^{-1} throughout the experiment (gas chromatographic analysis).

CH_4 consumption and CO_2 release rates

To determine net CH_4 and CO_2 exchange rates, replicate microcosms of all 30 communities were removed from the incubators and placed in air-tight 3 L jars with elevated CH_4 concentrations. Headspace samples were collected with a syringe and analyzed for CH_4 and CO_2 by gas chromatography (Agilent 7890N gas chromatograph; CH_4 was detected with a flame ionization detector; 12′ Porapak Q column; isothermic at 80°C; He carrier gas; CO_2 was determined on the same detector after reducing CO_2 with H_2 on a Ni-catalyst; Agilent Technologies Inc.,

Santa Clara, CA). A total of three headspace samples were collected over 3 days, except when CH_4 consumption rates were low, in which case three or four samples were taken over 4 days. Then, microcosms were placed back in the incubators. Gas exchange rates were calculated by linear regression of headspace concentrations against sampling time, with consumption rates converted to micromoles per microcosm per day by applying the ideal gas law.

DNA extraction

After 0, 31, 58, and 86 days of incubation, one of the replicates of each of the 15 dilution × dilution series combinations was removed from both incubators and DNA was retrieved using the xanthogenate-based extraction described in Schnyder et al. (2018). Extracted DNA was purified twice using the PowerClean Pro DNA Clean-Up Kit (MoBio, Carlsbad, CA) to remove PCR inhibitors.

Quantitative PCR (qPCR)

We quantified the size of total bacterial communities and methanotrophic communities by determining the abundances of the 16S rRNA gene (primers 27F and 1406R) and of the methanotrophic *pmoA* gene (subunit A of the particulate methane mono-oxygenase gene; primers A189F and mb661) by qPCR (StepOne real-time PCR system, Applied Biosystems, Foster City, CA; Appendix S1: Tables S1 and S2). For calibration, a serial dilution of purified DNA from *Methylococcus capsulatus* Bath (quantified with a Qubit Fluorometer, Invitrogen, Carlsbad, CA) was included in duplicate in each run. Additionally, we included reference samples with DNA of *M. capsulatus* on all plates and then standardized between plates using the geometric mean (Ruijter et al., 2015).

Sequencing

The composition of total bacterial and methanotrophic communities was determined by amplifying in duplicate the variable regions V1 and V2 of the 16S rRNA gene and the *pmoA* gene (primer pairs 27F/341R and A189F/mb661, respectively; Appendix S1: Tables S1 and S2). The duplicate PCR products were pooled and purified with the GeneJET PCR purification kit (Thermo Scientific, Waltham, MA) and quantified fluorometrically using the Qubit dsDNA BR Assay Kit (Thermo Fischer, Waltham, MA) on the Spark 10M Multimode Microplate Reader (Tecan, Männedorf ZH, Switzerland) with a standard curve (0–100 ng DNA μL^{-1}). Samples were barcoded with the Fluidigm Access Array technology and the amplified regions were paired-end sequenced on the Illumina MiSeq v3 platform at the Génome Québec Innovation Center, Montreal, Canada. Forward and reverse sequence data were merged, quality controlled and clustered by OTU (97% and 93% sequence similarity for 16S and *pmoA*, respectively) using a customized pipeline (Schnyder et al., 2018) that was based on the algorithms implemented in USEARCH v9 (Edgar, 2010).

To assess nonmethanotrophic OTUs using 16S data, we then removed all known methanotroph 16S sequences from this data set. The *pmoA* OTU sequences were blasted against the NCBI database and sequences that did not yield a match or were not *pmoA* were removed. For taxonomic classification, the OTU centroid sequences were mapped using the naïve Bayesian classifier implemented in MOTHUR (Schloss et al., 2009) with a minimum bootstrap support of 60%. $16S_{V1-V2}$ and *pmoA* sequences were mapped against sequences from the SILVA database (Pruesse et al., 2007), version 123, retrieved from https://www.arb-silva.de, and the *pmoA* taxonomic classifier (Dumont et al., 2014), respectively.

Alpha diversity and phylogenetic diversity

Phylogenetic trees (UPGMA) for the 16S rRNA gene and the *pmoA* gene were calculated with Clustal Omega (Sievers et al., 2011) using the web services from EMBL-EBI (McWilliam et al., 2013). *pmoA* phylogenies were built by adding sequences retrieved from GenBank for all accessions in the *pmoA* phylogeny in fig. 1 in Lüke and Frenzel (2011). The phylogenetic diversity (PD) of each sample was calculated as the total branch length of the phylogenetic subtree defined by the strains found in each sample (function calcPD; https://github.com/pascal-niklaus/pdiv). We randomly subsampled both 16S and *pmoA*-based OTU tables 5000 times to an even depth (10,000 sequences 16S rRNA, 18,000 sequences *pmoA*) and determined OTU richness (S), the Shannon's index of OTU distribution (H), and PD of these rarefied OTU sets. Rarefaction reduced S on average by 7% and 25% for *pmoA* and 16S, respectively. H was nearly invariant to rarefaction, with reductions <1%. Reductions in PD averaged 4% and 22% for *pmoA* and 16S, respectively. A few samples had too few sequences for rarefaction; this was the case in particular for *pmoA* in the 10^{-7} dilution at day 0. These samples are marked in figures and were excluded from statistical analyses.

Statistical analysis

We used analysis of variance (ANOVA) based on general linear models (R 4.1; http://r-project.org) and mixed models (ASReml-R 4; VSN International, Hemel Hempstead, UK) to test for effects of our experimental treatments on methanotrophic activity and community size. Fixed effects were the experimental treatment dilution (*log*-transformed), temperature (two-level factor), and their interactions. Community composition (the 15 specific combinations of dilution series and dilution) was included as a random effect. Because the goal of the dilution treatment was to reduce the diversity of methanotrophs, we fitted additional models with OTU diversity as explanatory variable. In our analyses, residuals generally were normally distributed and homogeneous except for the 10^{-7} dilution which sometimes showed enhanced variation; we accounted for this heteroscedasticity by fitting a heterogeneous random term ("idh" option of ASReml with a separate, larger, variance component for dilution 10^{-7}). Effects of temperature and dilution treatments on community composition were tested using permutational ANOVA (Andersen et al., 1998) as implemented in the "adonis" function of R-library "vegan," using Bray–Curtis dissimilarities among communities based on square-root-transformed OTU abundance data. To test for effect of the temperature treatments, data were permuted among temperature treatments within combinations of the dilution level and series. To tests for effects of dilution, dilution levels were permuted within dilution series, keeping pairs of microcosms with different temperature treatment as an unaltered unit of randomization. A principal coordinate analysis (PCoA) of Bray–Curtis dissimilarities was used to map community differences (β diversity) into two-dimensional space using the R function "cmdscale." These ordination plots were used to inspect the trajectories that dilution and time-series delineated in composition space, and whether trajectories depended on the temperature treatment.

RESULTS

Community diversity

We extracted DNA from microcosms after 0, 31, 58 and 86 days (end of the experiment) and sequenced the 16S rRNA gene (V1–V2) and the *pmoA* gene using the Illumina MiSeq platform. The 11×10^6 16S rRNA gene and 14×10^6 *pmoA* sequences clustered into 2697 nonmethanotrophic bacterial and 58 methanotrophic OTUs. OTU richness (S), Shannon's index of OTU abundances (H), and PD were statistically highly significantly related to dilution level (Figure 1; $F_{1,10} = 55$–274, depending on gene and diversity metric, $p < 0.001$ for all tests; Appendix S1: Table S3). Importantly, the established diversity gradient was maintained throughout the entire incubation, with similarly significant effects after 86 days. The realized diversity gradient was statistically independent of the temperature treatment (Appendix S1: Table S3).

Community composition

At the end of the experiment, the most common nonmethanotrophic bacterial taxa were Proteobacteria, Bacteroidetes, Actinobacteria and Verrucomicrobia. Type Ia methanotrophs, in particular *Methylobacter*, dominated the methanotroph community (Figure 2). Across all dilutions, the proportion of type Ia methanotrophs decreased with time, with opposite effects on type IIa methanotrophs (Figure 2). Conversely, low to intermediate dilution levels were characterized by high type Ia methanotrophs abundances, in particular of *Methylobacter* and *Methylosarcina*. At high dilution, the methanotroph community was almost exclusively composed of type IIa methanotrophs of the genera *Methylocystis* and *Methylosinus* (Figure 2).

PCoA of Bray–Curtis dissimilarities among communities revealed that composition changed relatively little with time but that the serial dilutions resulted in a consistent trajectory in ordination space, independent of the applied temperature treatment (Figure 3). In accordance, OTU composition was significantly affected by the dilution treatment, on all sampling dates, but the temperature had no such effect (PERMANOVA; Appendix S1: Table S4). Also, replicate communities became more dissimilar with dilution (mean Bray–Curtis dissimilarities among replicates; Appendix S1: Figure S1).

Community size

pmoA and 16S rRNA gene copy numbers were statistically independent of dilution (Figure 4a,b; Appendix S1: Table S5). Under cycling temperature, copy numbers were $\sim^1/_3$ lower than under constant temperature, for both 16S and *pmoA* ($F_{1,11} = 4.1$, $p = 0.07$ and $F_{1,11} = 8.1$, $p = 0.02$; Appendix S1: Table S5). At the beginning of the experiment (day 0), *pmoA* copy numbers were approximately two orders of magnitude lower in the highest dilution (10^{-7}) compared with less-diluted samples (Figure 4c), but this effect disappeared at the second sampling on day 31.

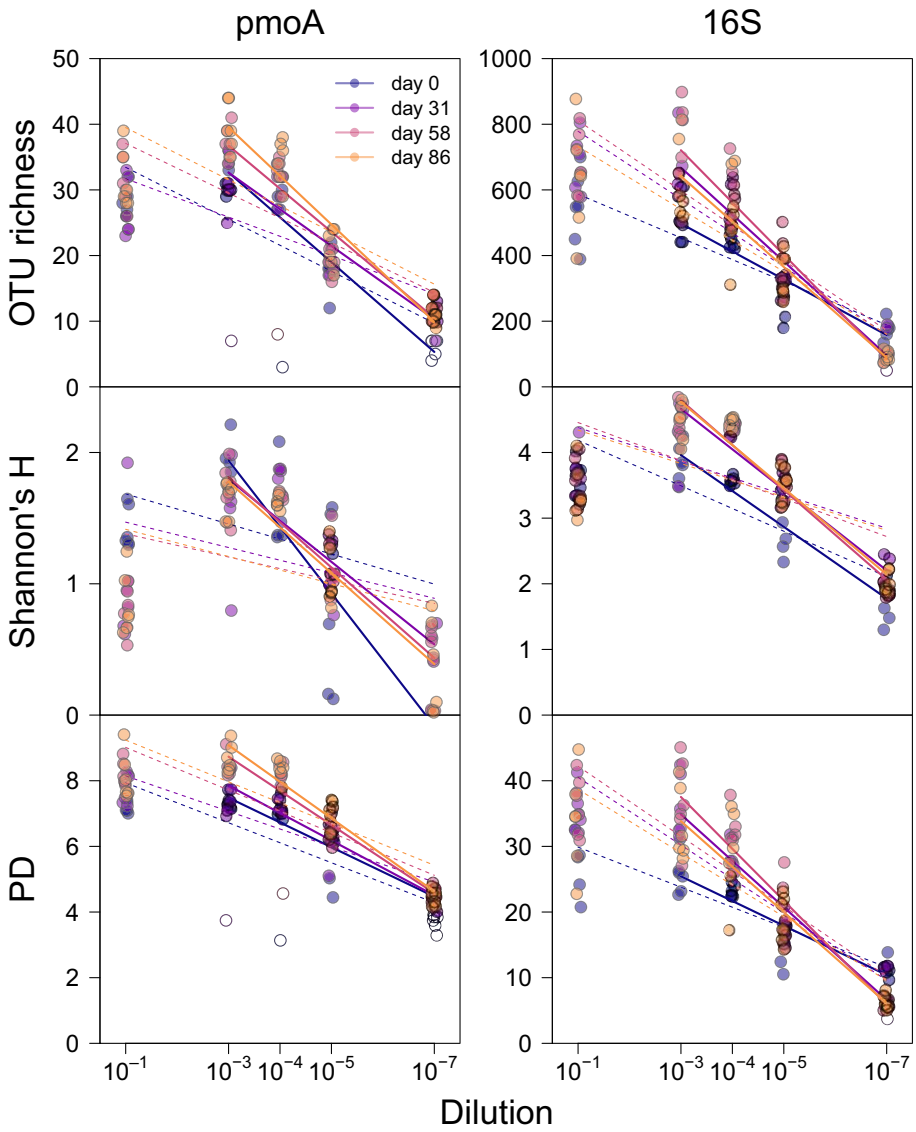

FIGURE 1 Effects of dilution on operational taxonomic units (OTU) richness, Shannon's diversity (H), and phylogenetic diversity (PD) in dependence of dilution level and sampling date. Diversity metrics are based on rarefied sequence sets. 16S OTUs do not include known methanotrophs, these were removed. Open symbols indicate samples for which no rarefaction was possible because sequence numbers were too low; these samples were not included in the computation of regression lines. DNA extraction was incomplete in the least diluted samples (10^{-1}) where a precipitate formed. Linear regressions therefore are provided excluding (solid lines) and including (dashed lines) the 10^{-1} dilution.

CH_4 consumption and CO_2 release rates

CH_4 consumption decreased 1.9-fold with diversity loss along the dilution gradient (Figure 5; $F_{1,11.4} = 74$, $p < 0.001$; Appendix S1: Table S6). Net CH_4 consumption was significantly related to *pmoA*-based methanotrophic OTU richness S, the Shannon's diversity index H, and PD (Figure 5; $p \leq 0.003$ for all diversity metrics; Appendix S1: Table S6). CH_4 consumption rates were higher initially and stabilized during the first 20 days, except for the highest dilution (10^{-7}) which showed no initial activity and then overshot before also stabilizing around day 30 (Figure 6). Toward the end of the experiment (days 60–86), diversity effects even grew larger, resulting in a 7.5-fold change from the 10^{-1} to the 10^{-7} dilution, with rates of 105 ± 9 and 14 ± 3 μmol CH_4 day^{-1} $microcosm^{-1}$, respectively. CO_2 production very closely mirrored CH_4 consumption ($r = 0.86$, Pearson's product-moment correlation, $p < 0.001$; data not shown). The temperature treatment did not affect net CH_4 consumption (Appendix S1: Table S6).

DISCUSSION

Using a dilution-to-extinction approach, we demonstrate that diversity loss can affect net methane (CH_4)

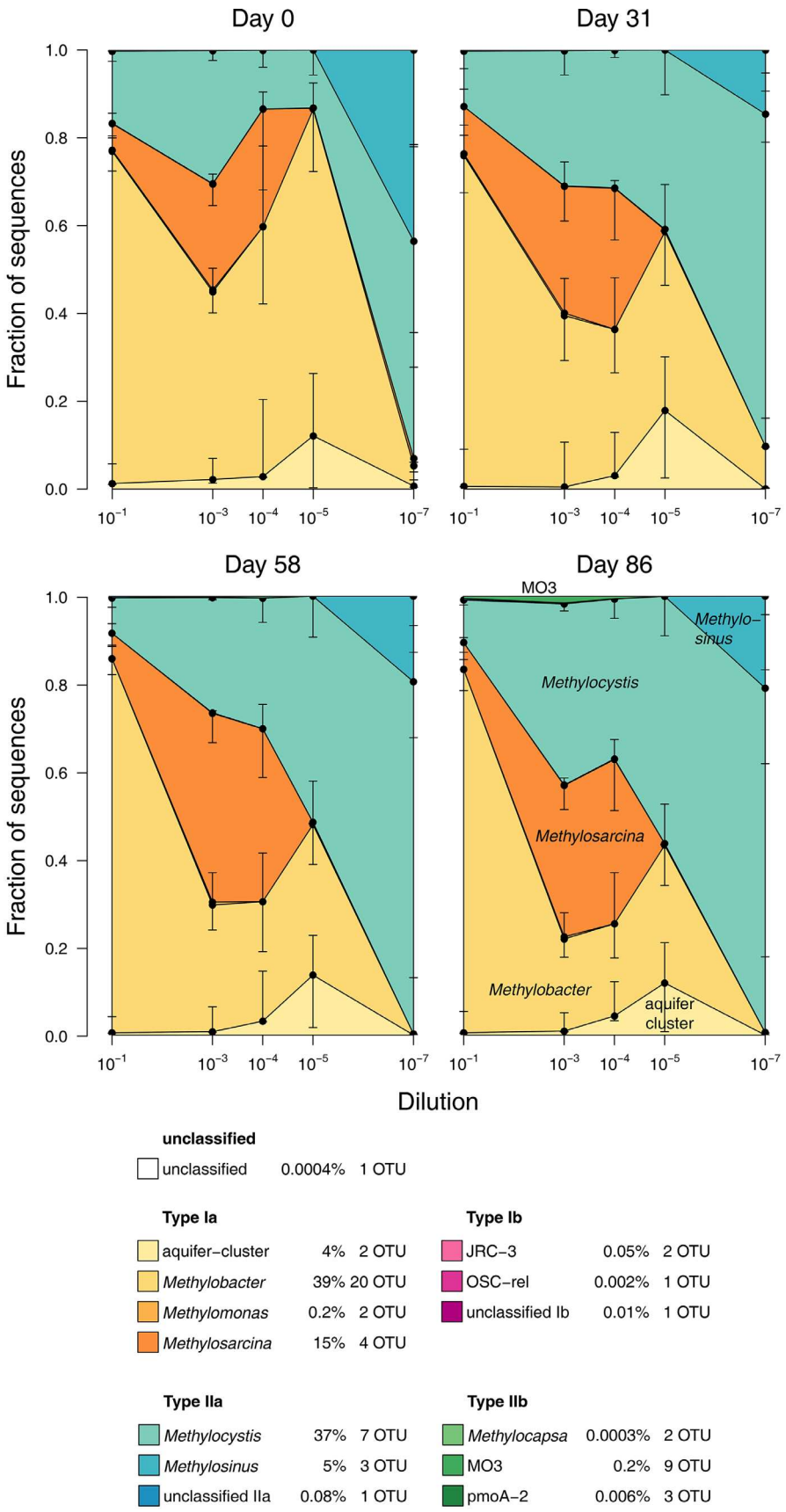

FIGURE 2 Effects of dilution on methanotroph community composition through time. The legend shows the average fractional abundance of all taxa across all sampling dates and dilution levels, together with the number of operational taxonomic units (OTU). Error bars show uncertainties for the different groups (1 SE) and are directed inwards.

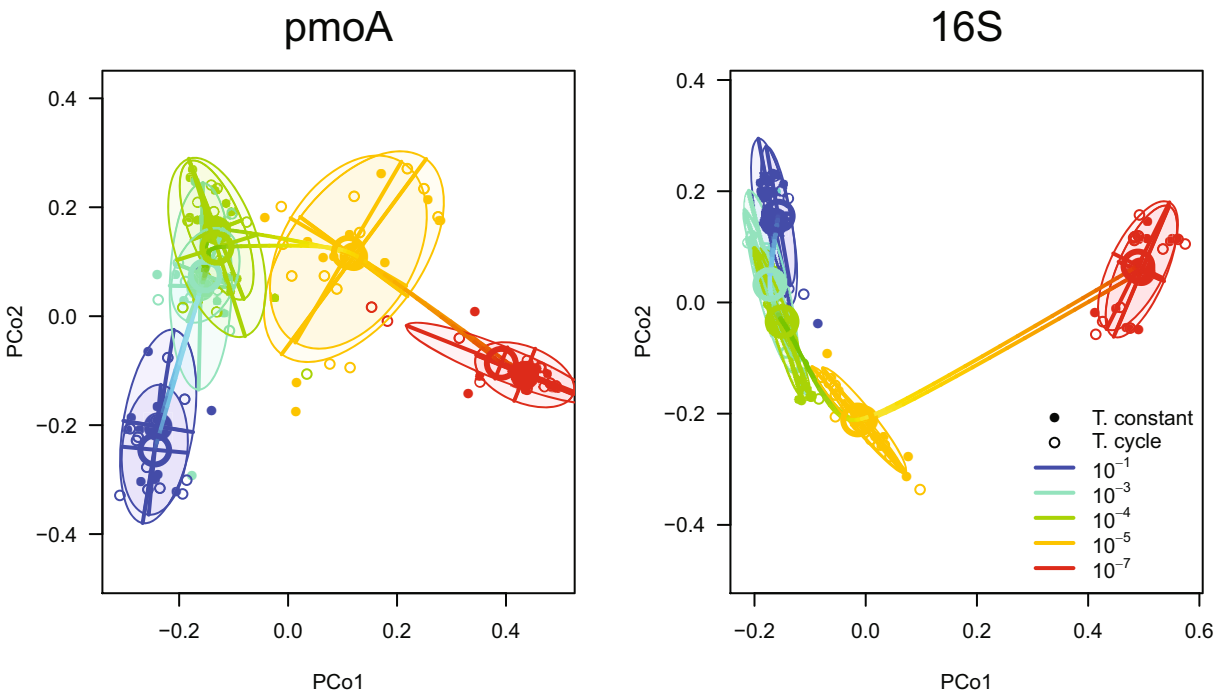

FIGURE 3 Community dissimilarities among the different dilution and temperature treatments. Ordination plots show principle coordinates of Bray–Curtis dissimilarities calculated from square-root-transformed *pmoA* and 16S sequence abundances. Ellipsoids indicate 67% confidence intervals. Known methanotrophic sequences were removed from 16S operational taxonomic units (OTU) data.

consumption by methanotrophic communities extracted from a landfill cover soil. Methanotrophic bacteria perform critical biogeochemical functions in many ecosystems; in landfills, including the one we studied here, methanotrophs typically capture nearly all CH_4 produced during the decomposition of waste material (Schroth et al., 2012). Landfills are an important anthropogenic source of CH_4. Establishing and managing cover soils to efficiently filter CH_4 is a key tool to mitigate landfill CH_4 emission. Our experiments suggest that this methanotroph-driven ecosystem service critically depends on the presence of a sufficiently diverse methanotrophic community.

Experimental research has shown that BEF relationships in communities of higher plants generally are positively decelerating; that is, the benefit of additional species decreases with the diversity of the system. These relationships may even saturate because of functional redundancy since the loss of a few species will functionally be compensated by others with no resulting net effect on community-level function (Salles et al., 2012). It has been argued that microbial communities are likely to exhibit pronounced functional redundancy (Nannipieri et al., 2003) due to their tremendous diversity and due to the relative simplicity of microbes compared with plants (Roesch et al., 2007; Sogin et al., 2006; Torsvik et al., 1990). If this were true, microbial diversity loss would only lead to a loss of function at the lower end of the diversity gradient (Nielsen et al., 2011). These considerations are highly relevant for methanotrophic communities, for several reasons. First, methanotrophs all perform the same specialized metabolic function that is restricted to comparably narrow phylogenetic groups. Second, methanotroph diversity typically is relatively low with rarely more than a few dozen OTUs. Third, methanotroph diversity and activity are vulnerable to disturbance, for example by high mineral N levels from fertilizer application (Bodelier & Laanbroek, 2004). In total, 58 methanotrophic OTUs were detected in the landfill cover soil under investigation. Although this number might appear high, we observed a clear reduction of CH_4 consumption rates under dilution-induced diversity loss, contrasting the notion that functional redundancy protects ecosystem functioning from diversity loss. Importantly, these detrimental effects manifested despite the fact that the eliminated OTUs were of low abundance. This finding corroborates earlier studies that used isotope probing of methanotrophic communities and found that subordinate taxa contributed significantly to community-level functioning (Bodelier et al., 2013).

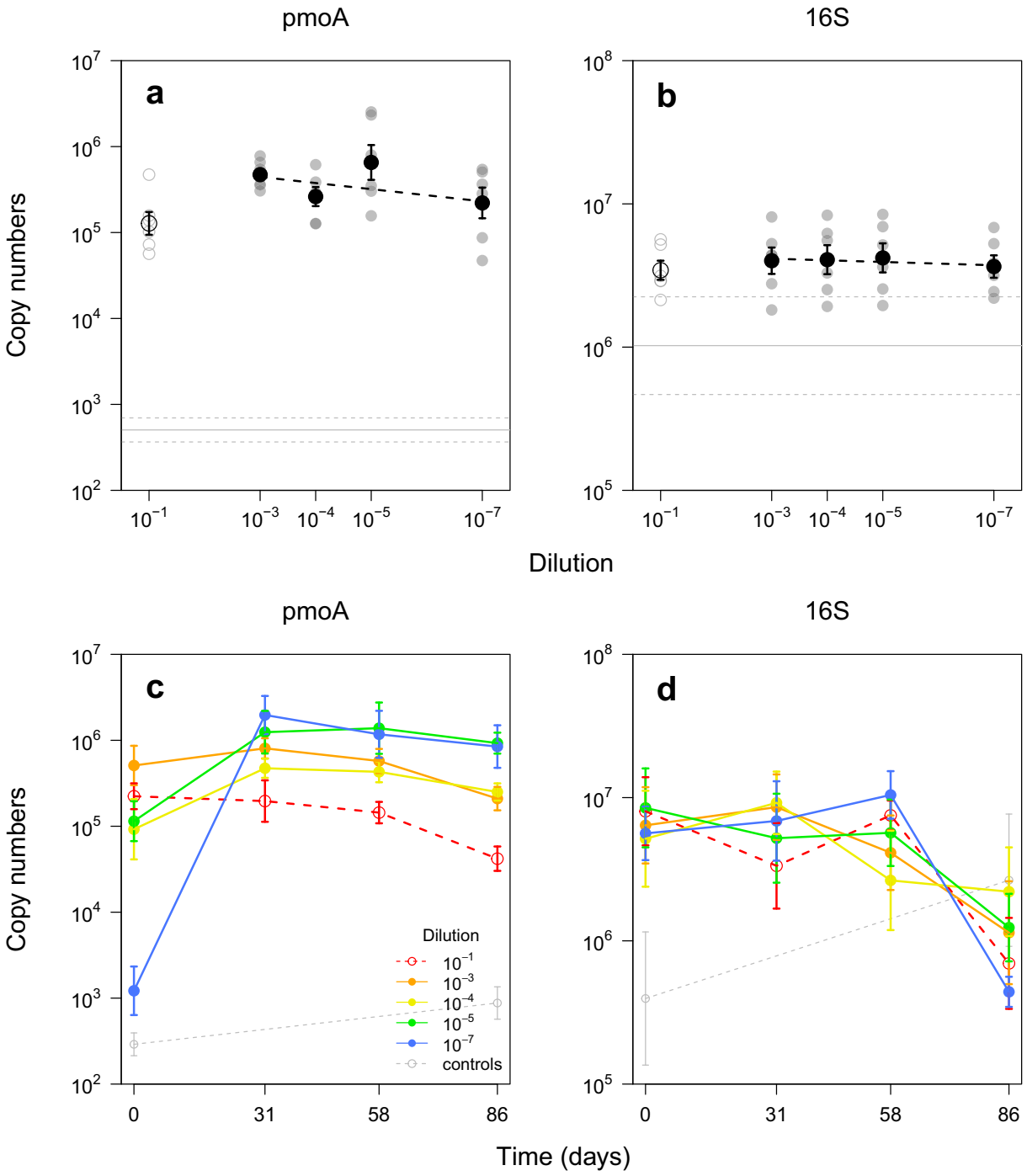

FIGURE 4 Total bacterial and methanotroph community size, determined as (a, c) *pmoA* and (b, d) 16S rRNA gene copy numbers. (a, b) Gene copy numbers in dependence of applied dilution treatment. Gray symbols indicate averages across four sampling dates for each community composition × temperature treatment combination (3 dilution series × 5 dilution levels × 2 temperatures). Dashed lines show linear regressions accounting for heterogeneous variance among dilution levels. Black symbols indicate mean copy numbers for each dilution level (mean ± SE; $n = 6$). (c, d) Gene copy numbers in dependence of sampling date and dilution (mean ± SE; $n = 6$). In all figures, the gray lines in the lower part indicate gene copy numbers in sterile control microcosms (mean ± SE; $n = 4$).

The use of serial dilutions to inoculate sterile soil microcosms is the method of choice to reduce diversity in microbial BEF studies where the number of species is high and their identity unknown (Garland & Lehman, 1999; Yan et al., 2015). Dilution preferentially removes rare and slowly recovering taxa (Giller et al., 2004). In our study, the less-diluted samples were dominated by type II methanotrophs, reflecting the conditions found in the

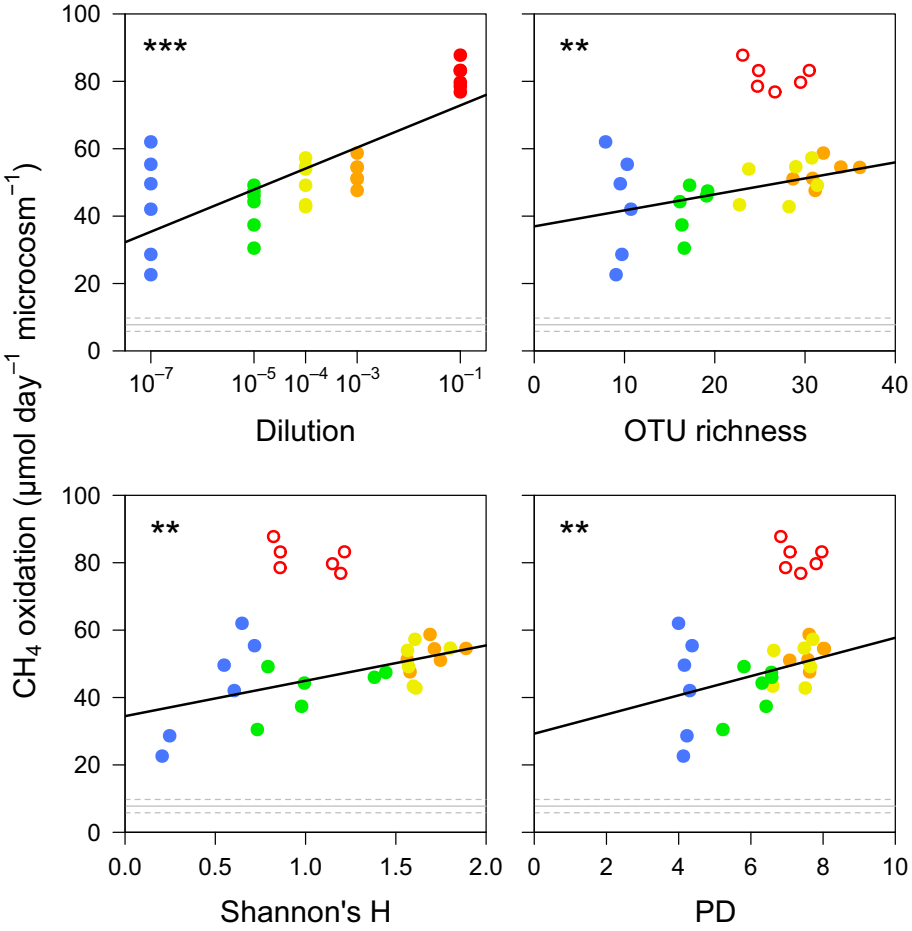

FIGURE 5 Net CH_4 consumption rates of microcosms in dependence of dilution level and methanotrophic diversity (*pmoA*-based richness of operational taxonomic units (OTU), Shannon index [H], and phylogenetic diversity [PD]). Symbols show means for each microcosm ($n = 3$ replicates × 2 temperature treatments per dilution level; temperature: n.s.). Samples with 10^{-1} dilution are excluded from statistical tests (indicated by open symbols) because of incomplete DNA extraction due to precipitate. Gray lines indicate rates for sterile control microcosms (mean ± SE; $n = 4$). ***$p < 0.001$; **$p < 0.01$; *$p < 0.05$.

original landfill cover soil (Henneberger et al., 2012). As communities kept growing over the course of the experiment, the type I methanotrophs *Methylocystis* and *Methylosinus* became more dominant. The same shift toward type I methanotrophs, but much more pronounced, was found in the highest dilution (10^{-7}) for which larger growth was required for communities to recover from dilution. The reasons for this shift remain unknown. It may be that the original dominance by type II methanotrophs would have been restored had some steady state been reached if the experiment had been continued for long enough. In our study, the applied serial dilutions were highly correlated with OTU-based diversity metrics for methanotrophic and nonmethanotrophic bacterial communities, but the 10^{-1} dilution deviated from the overall pattern. In this dilution (but not in others), a milky residue and gel-like pellets occurred during DNA extraction and probably reduced DNA yields and led to a relative underestimation of diversity. The primary treatment applied, dilution level, was unaffected by this artifact and explained the observed responses better than the OTU-based metrics unless the least diluted treatment was excluded. Irrespective of this methodological caveat, the relationship between diversity and methanotrophic activity was robust, resembling patterns of BEF relationships found in plant communities (Cardinale et al., 2012).

In contrast with diversity experiments in which artificial communities are assembled de novo from isolated strains (Schnyder et al., 2018), dilution treatments decrease the abundance of both target and nontarget microorganisms, and our study was no exception. However, soil communities recovered well during the 4-week preincubation, with no significant differences in total bacterial and methanotrophic community size remaining at day zero except for the most diluted community, which required more time to recover. Nonmethanotrophic bacterial community size decreased

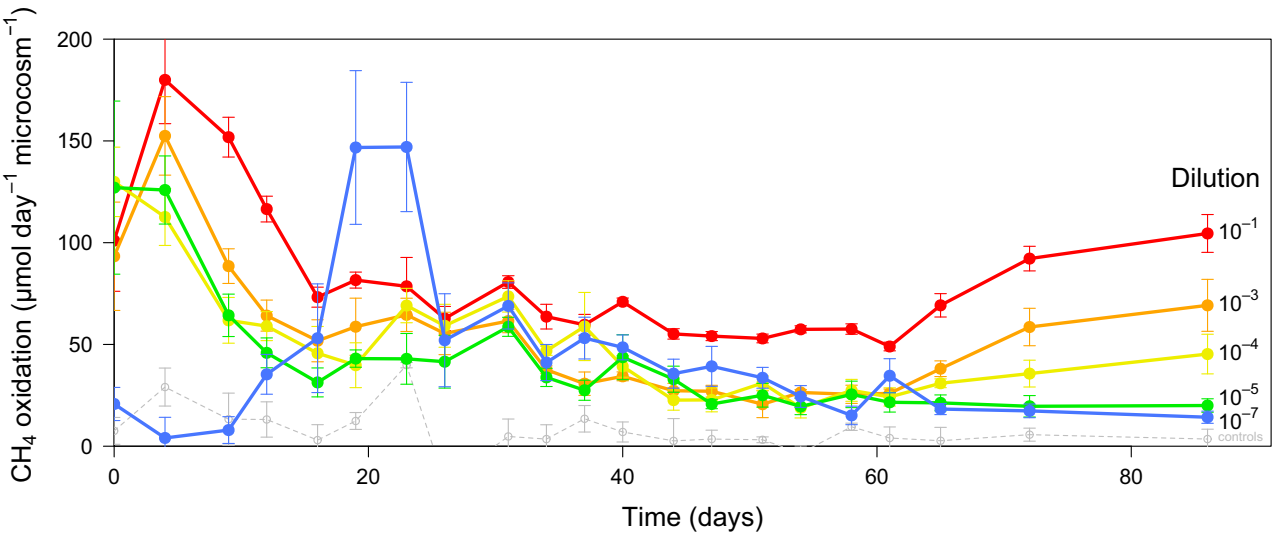

FIGURE 6 Net CH_4 consumption rates of microcosms as a function of dilution level and sampling date. Data of the two temperature treatments were combined because no temperature effect was detected. Gray lines indicate rates in sterile control microcosms (mean ± SE; $n = 4$). Symbols show mean ± SE ($n = 6$).

over the course of the experiment, but this change was independent of dilution. Methanotroph community size showed dilution-dependent temporal dynamics, with more diluted samples displaying higher initial growth rates. This was probably the case because methanotroph abundances were below the carrying capacity of the system, possibly combined with reduced competition for oxygen and nutrients by heterotrophs. As a result, methanotrophs initially grew exponentially and community size overshot in microcosms of the highest dilution treatment. A similar effect has been observed in other studies, including sewage microbial communities (Franklin et al., 2001) and grassland denitrifier communities (Philippot et al., 2013), in which the highest dilutions showed the strongest initial growth in bacterial abundance. However, in our study, this effect was transient, with trends in community size that reversed toward the end of the study. Despite the temporal dynamics in community size, the effects of dilution and OTU diversity on CH_4 consumption remained remarkably stable, indicating that the BEFeffects found were unrelated to the initial fluctuations in community size.

Toward the end of the experiment, net CH_4 consumption increased and biodiversity effects became most pronounced. This parallels many studies with plant systems in which BEF relationships grew larger through time (Cardinale et al., 2007; Huang et al., 2018; Marquard et al., 2009; Reich et al., 2012; van Moorsel et al., 2018). The methanotrophs in our study thrived on above-atmospheric CH_4 concentrations, displayed an apparent low-affinity kinetic (Dunfield, 2007), and had a relatively short generation time compared with plants; our three-month experiment under near-optimal growth conditions will thus have allowed for many generations. BEF effects that grew through time also have been observed in other microbial systems (Fiegna et al., 2015; Gravel et al., 2011), although patterns generally varied among environments. We did not investigate the specific mechanisms that promoted biodiversity effects; however, there is evidence that stronger BEF relationship can emerge from the evolution of less negative species interactions (Fiegna et al., 2015) or from an increase in interspecific niche complementarity (microbes: Lawrence et al., 2012, plants: Zuppinger-Dingley et al., 2014).

Methanotroph diversity may buffer ecosystem functioning during environmental fluctuations (Collet et al., 2015; Ho, Angel, et al., 2016; Ho, van den Brink, et al., 2016). Methanotrophs isolated from environmental samples typically exhibit distinct temperature optima. We therefore expected stronger BEF relationships in the model communities that were exposed to a diurnal temperature cycle because we reasoned that more diverse communities would span a wider range of temperature optima. However, we did not find such an effect. One reason may be that we assessed CH_4 consumption outside the incubator, at constant temperature, regardless of the original treatment. We nevertheless would have expected that effects present in the incubator would have persisted for some time and therefore would have been detectable in the measurements. Interestingly, we also did not observe any effect of temperature regime on the diversity of methanotrophs or their specific community composition. This implies that these communities remained unaffected by temperature cycling, possibly because the temperature

variability resembled the fluctuations found naturally in the original landfill soil and species or entire communities were adapted to these conditions.

The food web in our microcosm communities was largely CH_4 based, with no plants present in the microcosms and plant cover was relatively low at the original landfill sampling site. However, our dilution-to-extinction approach reduced not only the diversity of methanotrophs but also the one of nonmethanotrophic bacteria (and potentially also of other groups of organisms that we did not measure, for example protists). In natural systems, the diversity of even remote taxa often co-varies (Oehri et al., 2017; Wolters et al., 2006), so that BEF relationships observed in such systems reflect the compound effects of diversity changes across multiple, potentially interacting, taxonomic groups. Methanotrophic growth is often stimulated by the presence of other microorganisms, most likely because these provide co-factors or remove metabolites that otherwise accumulate to inhibitory levels (Ho et al., 2014; Ho, Angel, et al., 2016). Recent experiments have also shown that volatiles produced by heterotrophs, specifically dimethyl sulfides, can affect methanotrophic growth even when heterotrophs are physically isolated (Veraart et al., 2018). Although we think that the reduced methanotrophic activity resulted primarily from a loss of methanotrophic strains, it is possible that a loss of interactions with heterotrophic bacteria further exacerbated the reduced functioning in low-diversity communities.

Methanotrophs play a key role in the regulation of climate by oxidizing large fractions of the CH_4 produced in anaerobic environments, thereby preventing CH_4 emissions. Methanotrophs also oxidize significant amounts of atmospheric CH_4 (IPCC, 2021). Our results indicate that a reduction in the diversity of methanotrophs can affect system-level CH_4 consumption through mechanisms independent of community size. The past years have seen an accelerated rise in atmospheric CH_4 loads (Mikaloff Fletcher & Schaefer, 2019), which underlines the critical importance of diverse methanotrophic communities in sustaining stable CH_4 oxidation services in managed and natural wetlands. More generally, our study suggests that microbial diversity supports microbially driven ecosystem functions in a way similar to the one found in plants; that is, positive BEF relationships appear universal across very different taxa and spatial scales.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

Data and code (Schnyder et al., 2022) are available in Zenodo at https://doi.org/10.5281/zenodo.7520706. Sequence data are deposited in the European Nucleotide Archive under accession no. PRJEB64145 at https://www.ebi.ac.uk/ena/browser/view/PRJEB64145.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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