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ARTICLE

Biological H_2 and CO oxidation activities are sensitive to compositional change of soil microbial communities

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Abstract: Trace gas uptake by microorganisms controls the oxidative capacity of the troposphere, but little is known about how this important function is affected by changes in soil microbial diversity. This article bridges that knowledge gap by examining the response of the microbial community-level physiological profiles (CLPPs), carbon dioxide (CO_2) production, and molecular hydrogen (H_2) and carbon monoxide (CO) oxidation activities to manipulation of microbial diversity in soil microcosms. Microbial diversity was manipulated by mixing nonsterile and sterile soil with and without the addition of antibiotics. Nonsterile soil without antibiotics was used as a reference. Species composition changed significantly in soil microcosms as a result of dilution and antibiotic treatments, but there was no difference in species richness, according to PCR amplicon sequencing of the bacterial 16S rRNA gene. The CLPP was 15% higher in all dilution and antibiotic treatments than in reference microcosms, but the dilution treatment had no effect on CO_2 production. Soil microcosms with dilution treatments had 58%–98% less H_2 oxidation and 54%–99% lower CO oxidation, relative to reference microcosms, but did not differ among the antibiotic treatments. These results indicate that H_2 and CO oxidation activities respond to compositional changes of microbial community in soil.

Key words: microbial ecology, molecular hydrogen, carbon monoxide, trace gas.

Résumé : Le captage de gaz à l'état de traces par les microorganismes contrôle la capacité d'oxydation de la troposphère, mais on sait peu de choses sur la façon dont cette fonction importante est affectée par les changements de la diversité microbienne du sol. Cet article comble les lacunes dans les connaissances en examinant la réponse à la manipulation de la diversité microbienne dans les microcosmes du sol quant au profil physiologique de la communauté microbienne (CLPP), à la production de dioxyde de carbone (CO_2) et à l'oxydation d'hydrogène moléculaire (H_2) et de monoxyde de carbone (CO). La diversité microbienne a été manipulée en mélangeant des sols non stériles et stériles, avec ou sans ajout d'antibiotiques. Le sol non stérile sans antibiotiques a été utilisé comme référence. La composition en espèces changeait de manière significative dans les microcosmes du sol à la suite de la dilution et des traitements aux antibiotiques, mais il n'y avait pas de différence quant à la richesse en espèces selon le séquençage d'amplicons PCR du gène bactérien de l'ARNr 16S. Le CLPP était 15 % plus élevé dans tous les sols dilués et tous les sols traités aux antibiotiques comparativement aux microcosmes de référence, mais la dilution n'avait pas d'effet sur la production de CO_2. Les microcosmes des sols dilués présentaient de 58 à 98 % moins d'oxydation de H_2 et de 54 à 99 % moins d'oxydation de CO relativement aux microcosmes de référence, mais ne différaient pas en ce qui concerne les traitements aux antibiotiques. Ces résultats indiquent que les activités d'oxydation de H_2 et de CO répondent aux changements de composition de la communauté microbienne du sol. [Traduit par la Rédaction]

Mots-clés : écologie microbienne, hydrogène moléculaire, monoxyde de carbone, gaz à l'état de traces.

Introduction

Soil is a heterogeneous environment where aboveground biomass, voids in the surrounding aggregates, and uneven water availability shape various nutrient, physical, and chemical gradients (Lehmann et al. 2008; Vos et al. 2013; Young and Crawford 2004). The mosaic landscape of soil exerts environmental filtering on microbial communities; communities demonstrate remarkable

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metabolic flexibility and adaptation capabilities, as evidenced by the 10 000 species contained in 1 g of soil (Curtis and Sloan 2004; Rosselló-Mora and Amann 2001). The importance of microbial diversity in securing ecosystem services in soil is complex due to the disproportional contribution of rare species and functional redundancy in microorganisms (Jousset et al. 2017).

Direct manipulation of microbial diversity is a way to determine how a particular function is responding to changes in diversity. This can be done by assembling an artificial microbial population or by reducing the inherent diversity with fumigation or dilution to extinction methods. Application of these methods has shown that the productivity of the function is impacted differently, depending on whether specialized functions, such as recalcitrant carbon degradation (Maron et al. 2018) and denitrification (Philippot et al. 2013), or a broad function, such as carbon dioxide (CO_2) production (Langenheder et al. 2006) and labile carbon degradation (Baumann et al. 2013), is examined.

The sensitivity of microbial uptake of trace gases to an alteration in soil microbial diversity is underrepresented in the literature, despite microorganisms' mitigation of natural and anthropogenic emissions of climate-relevant trace gas(es) in the atmosphere. For instance, the soil uptake of atmospheric molecular hydrogen (H_2) and carbon monoxide (CO) involving specialized high-affinity H_2-oxidizing bacteria (HOB) and high-affinity CO-oxidizing bacteria (COB) is responsible for 80% and 15% of the global attenuation of H_2 and CO, respectively (Novelli et al. 1998, 1999). These microbial processes are of critical importance in reducing the atmospheric lifetimes of H_2 and CO for which the global burden modulates the oxidative capacity of the atmosphere. HOB are unevenly distributed in *Actinobacteria*, *Proteobacteria*, *Chloroflexi*, and *Acidobacteria* (Constant et al. 2010; Greening et al. 2014, 2015; Islam et al. 2019), whereas COB are members of the *Proteobacteria*, *Actinobacteria*, and *Chloroflexi* (King and Weber 2007). Environmental HOB and COB are poorly represented in public genome databases but could represent 1% and 3% of the soil bacterial community, respectively, based on their relative abundance in qPCR assays (Khdhiri et al. 2015; Lalonde and Constant 2016).

This study tested the sensitivity of H_2 and CO uptake to manipulation of microbial diversity in soil microcosms subjected to dilution (three dilution ratios of sterile and nonsterile soil) and the addition of antibiotics. Generalist functions of CO_2 production and the community-level physiological profiles (CLPPs) of the saprophyte fungi and heterotrophic bacteria were also monitored. The dilution and antibiotic treatments were expected to alter the composition but not the species richness of the soil microbial communities. Changes in H_2 and CO oxidation, as well CO_2 production and the CLPP, were related to the taxonomic composition of soil bacterial communities (based on 16S rRNA gene PCR amplicon sequencing) after 21, 28, 35, and 42 days of incubation.

Materials and methods

Soil samples

Three soil samples were collected with a shovel in a sugar maple forest (45.55 N; 73.73 W), one each in April, July, and September of 2017, respectively. The upper layer (10 cm) of the B horizon was collected after removal of the litter, fermented, and humus soil horizons (upper 2 to 5 cm layer). Soil collected on each date was stored at 4 °C for a few days and then homogenized and sieved (<2 mm) with a vibratory sieve shaker (AS 200; Retsch GmbH, Haan, Germany). Each of the three samples was then partitioned into two subsamples: the first was designated as "soil inoculum" and was stored at 4 °C, and the second was designated as "sterile soil substrate" and was stored at –20 °C after sterilization by Cobalt 60 irradiation with a 50 kGy dose (Nordion, Laval, Quebec, Canada), eliminating most soil bacteria (McNamara et al. 2003). Soil incubation began within a week after sieving.

Preparation and incubation of soil microcosms

Soil microcosms (160 g) were prepared by mixing soil inoculum and sterile soil substrate at three ratios (1/4, 1/20, and 1/40). A fixed amount of conditioned 0.5-mm-diameter glass beads (40 g) was added to each microcosm. Beads were conditioned by adding 13 mL of methanol with or without carbenicillin (11 $mg \cdot mL^{-1}$) plus tetracycline (12 $mg \cdot mL^{-1}$) antibiotic mixture to the bead aliquots, which were subsequently thoroughly mixed and dried for a 12 h period in a chemical fume hood. Conditioned glass beads were used as the vector for carbenicillin (118 $mg \cdot kg^{-1}$) plus tetracycline (125 $mg \cdot kg^{-1}$) in soil (Thiele-Bruhn and Beck 2005). Preliminary experiments demonstrated that the antibiotic dose exerted no significant effect on H_2 and CO oxidation activities in soil, while soil respiration increased by a factor of two (data not shown). Soil microcosms containing 160 g of soil inoculum and 40 g of conditioned glass beads with methanol were included as "reference microcosms" in the experiment. A total of seven microcosms (three soil dilutions × two antibiotic treatments (i.e., with, without) and one reference) were set up. The entire experiment was repeated three times, with three different incubations (blocks) involving independent soil samples collected in the field for a total of 21 microcosms (seven microcosms × three blocks).

Soil microcosms were incubated in cell culture flasks (Falcon 225 cm^3) exposed to a dynamic headspace where the air composition reflected the atmospheric concentration of trace gases (Khdhiri et al. 2017). There were two stages in the incubation: the pre-incubation stage (days 1 to 20), aimed at establishing equivalent bacterial biomass between each microcosm, and the sampling stage (days 21, 28, 35, and 42), dedicated to microbial activity measurements and DNA extraction. Microcosm soil sam-

ples collected for DNA extraction (0.5 g) were transferred to a 2 mL microtube containing 0.5 g of glass beads (0.15–0.21 mm diameter), 1 mL of TEP buffer (Krsek and Wellington 1999), and 20 μL of 20% sodium dodecyl sulfate. Tubes were stored at –20 °C until the DNA extraction procedure. Soil water content was adjusted to 25% water holding capacity and monitored every 2–3 days during the incubation by weighing the microcosms. Sterile water was added to maintain a constant soil moisture level in each microcosm.

Measurement of microbial processes

High-affinity H_2 and CO oxidation rates were measured using a gas chromatograph (Ametek, Berwyn, Pennsylvania, USA) equipped with a reduction gas detector (Khdhiri et al. 2015). Briefly, soil subsamples (10 g) were collected from microcosms and transferred in 500 mL sterile Wheaton glass bottles. A screw-cap fitted with a septum enabled successive injections of defined volumes (3 mL) of a certified gas mixture containing 520 ± 10 parts per million by volume (ppmv) H_2 and a second certified gas mixture containing 510 ± 10 ppmv CO (Distribution Praxair Canada, Saint-Laurent, Quebec, Canada) in the static headspace. First-order oxidation rates were computed by integrating H_2 and CO time series recorded over 6 h. The net CO_2 production rate was measured using a gas chromatograph (Agilent Technologies, Santa Clara, California, USA) equipped with a flame ionization detector (Khdhiri et al. 2017). Briefly, glass bottles containing 10 g subsamples were closed with a screw-cap fitted with a septum. Gas samples were collected from the static headspace to compute linear increase of CO_2 concentrations over 72 h. Finally, CLPPs were obtained using EcoPlates assay (Biolog, Hayward, California, USA). The procedure was the same as described in a previous investigation (Khdhiri et al. 2017), except that the incubation time was 7 days instead of 4 days. Patterns of the CLPPs were expressed using the Shannon diversity index. For each of the three incubations, microbial processes were measured in the seven microcosms at days 21, 28, 35, and 42. The H_2 and CO oxidation rates, CO_2 net production rates, and CLPPs presented in this article are proportional activities unless otherwise stated. Proportional activity was computed at each sampling date by dividing the process rate or Shannon diversity index measured in the microcosms subjected to diversity manipulation treatments by the value observed in reference microcosm. A ratio above 1 indicated an enhanced microbial process in response to treatment when compared with the reference microcosm. Raw data of process rate measurements are provided in supplemental Table S1[1].

DNA extraction

Frozen soil subsamples dedicated to DNA extraction were thawed at room temperature before the application of mechanical lysis at 6.5 $m \cdot s^{-1}$ for 45 s with the FastPrep-24 Sample Preparation System (MP Biomedicals, Santa Ana, California, USA). DNA was purified through successive phenol–chloroform (pH 7.0) and chloroform (pH 7.0) extractions and precipitation in 95% ethanol. Each pellet was dried at room temperature under aseptic conditions and suspended in 100 μL of nuclease-free sterile water. The extracted DNA was purified following the protocol of the OneStep PCR Inhibitor Removal kits (Zymo Research, Irvine, California, USA). The integrity of DNA was visualized using 1% agarose gel electrophoresis.

qPCR of bacterial 16S rRNA gene

The abundance of bacterial 16S rRNA gene copies in soil was determined by qPCR with the primers Eub-338 and Eub-518 (Fierer et al. 2005). The PCR-amplified 16S rRNA gene from *Escherichia coli* was used to generate a standard curve with the forward primer PA-27f-YM (Frank et al. 2008) and reverse primer PH (Fernández et al. 1999). Serial dilutions of the purified PCR product were done to obtain a standard curve ranging from 1×10^9 to 1×10^1 copies of the strand matrix ($R^2 = 0.99$ and reaction efficiency from 0.9 to 1.1). Template DNA was diluted in nuclease-free water (1:100) prior to qPCR performed using a PerfeCTa SYBR Green FastMix kit (Quanta Bio, Beverly, Massachusetts, USA). The thermocycler program comprised an initial denaturation step at 95 °C for 5 min, followed by 40 cycles, including a successive denaturation step at 95 °C for 45 s, an annealing step at 55 °C for 45 s, and an elongation step at 72 °C for 45 s. Fluorescence data were read during the elongation phases. A melting curve completed the reaction from 72 to 99 °C with a hold of 5 s.

PCR amplicon sequencing of bacterial 16S rRNA gene

Composition of soil bacterial communities was examined using genomic DNA extracted from the 21 microcosms at days 21, 28, 35, and 42 (total of 84 DNA extracts). PCR amplifications necessary for the preparation of sequencing libraries were conducted with high-fidelity AccuPrime *Taq* DNA Polymerase (Invitrogen, Carlsbad, California, USA). The V6–V8 region of bacterial 16S rRNA gene was first PCR amplified using modified B969F and BA1406R primers (Comeau et al. 2010) with the 5′ linker sequence 5′-TCGTCGGCAGCGTCAGATGTGTATAAGAGACAG-3′ and 5′-GTCTCGTGGGCTCGGAGATGTGTATAAGAGACAG-3′ for forward and reverse primers, respectively. PCR products were verified by agarose gel migration followed by a PCR clean-up using AMPure XP beads (Beckman Coulter, Brea, California, USA). Purified PCR products were sub-

[1]Supplementary data are available with the article through the journal Web site at http://nrcresearchpress.com/doi/suppl/10.1139/cjm-2019-0412.

jected to a second PCR performed for the library preparation using a Nextera XT kit (Illumina, San Diego, California, USA). PCR products were verified by agarose gel migration followed by a PCR clean-up using AMPure XP beads (Beckman Coulter). PCR amplicons were then quantified using Quant-iT PicoGreen dsDNA assay (Invitrogen), diluted to 1.5 ng·μL^{-1} and pooled together before shipping for sequencing. PCR amplicons were sequenced on an Illumina MiSeq PE-250 platform at the McGill University and Genome Quebec Innovation Center.

Raw sequencing reads were processed using the software Usearch version 10 (Edgar 2010). Paired reads were assembled to a total length varying from 400 to 500 nt, and then merged sequences were subject to quality control. Maximum mismatch threshold in the overlapped region of the assembly was set at 5, and primers were removed from each sequence. Sequences having 1 or fewer erroneous bases were accepted for downstream quality control steps. Reads were then dereplicated, and singletons were discarded and denoised using Unoise 3 (Edgar 2016). Sequences shorter than 366 nt were discarded. Filtered sequences were clustered into amplicon sequence variants (ASVs) displaying 100% identity (Callahan et al. 2017). ASVs representing <0.005% of read counts were removed. Taxonomic affiliation of the ASVs was assigned by the *k*-mer similarity of ASV representative sequences to the Ribosomal Database Project version 16 training set of 16S rRNA gene for bacteria (Cole et al. 2013). Owing to uneven distribution of the library size, which varied from 2 to 253 967 reads, only the 36 libraries comprising 10 000 to 50 000 reads were kept for downstream analyses (supplemental Table S1[1]). Raw sequence reads were deposited in the Sequence Read Archive of the National Center for Biotechnology Information under Bioproject PRJNA543329.

Statistical analysis

Statistical analyses were performed using the software R version 3.5.1 (R Core Team 2013). Microbial processes measured in microcosms subjected to soil dilution and antibiotic treatments were standardized with observed activity in reference microcosms. Under this framework, proportional activity above 1.0 indicated an enhancement of microbial process in response to treatments when compared with reference microcosms containing nonsterile soil without antibiotics. The hypothesis that the distribution of proportional activities in each treatment would be different from 1.0 was tested by Wilcoxon tests with the package Stats version 3.5.1. α-Diversity based on the first three Hill numbers, $q = 0$ (species richness), $q = 1$ (the exponential of Shannon's entropy index), and $q = 2$ (the inverse of Simpson's concentration index), was computed with the package iNEXT version 2.0.18 (Hsieh et al. 2016). The three Hill numbers were interpolated at 5541 reads, corresponding to half of the number of reads obtained in the smallest sequencing library (Chao et al. 2014). The impact of dilution and antibiotic treatments on the three α-diversity indicators and the abundance of 16S rRNA gene copies per gram of soil dry mass were compared with Kruskal–Wallis test followed by a Dunn post hoc test with the package dunn.test version 1.3.5 (Dinno 2017). Hellinger transformation was applied on the sparse ASV table (Legendre and Gallagher 2001) before the ordination of bacterial community profiles in a reduced space constrained by a principal coordinate analysis (PCoA). The PCoA was generated with the packages Phyloseq version 1.24.2 (McMurdie and Holmes 2013), ggplot2 version 3.0.0 (Wickham 2016), and plyr version 1.8.4 (Wickham 2011). The impact of soil dilutions, antibiotics, and blocks on β-diversity was tested with the comparison of Bray–Curtis distance using a permutational multivariate analysis of variance (PERMANOVA) with the package Vegan version 2.5-2 (Oksanen et al. 2013). Results of the PERMANOVA were presented in a Venn diagram done with the package VennDiagram version 1.6.0 (Chen and Boutros 2011). Linear regression analyses explaining the variation of proportional activities with the average Bray–Curtis dissimilarity of bacterial community profiles separating each microcosm from reference microcosms were performed with the package Stats version 3.5.1.

Results

Microbial processes in soil

CO and H_2 oxidation rates measured in the reference microcosms were in the range of 24 ± 15 and 26 ± 13 nmol·$g_{(soil\text{-}dm)}^{-1}$·$h^{-1}$, respectively. The relative H_2 and CO oxidation activities of each dilution and antibiotic treatment were significantly lower than the 100% activity displayed by the reference microcosms (Figs. 1A–1B). CO_2 production measured in the reference microcosms was in the range of 20 ± 6.2 nmol·$g_{(soil\text{-}dm)}^{-1}$·$h^{-1}$. Respiration rates were not distinguishable between the reference and the three respective dilution treatments (Fig. 1C), albeit antibiotics led to a significant enhancement of the activity in the 1/20 and 1/40 dilutions. The CLPP expressed as the Shannon index was 2.88 ± 0.19 in the reference microcosms. Intensification of the activity was observed in all dilution and antibiotic treatments, without a difference among the treatment groups (Fig. 1D). These results showed that the soil dilution and antibiotic treatments affected the generalist processes involved in CO_2 production and CLPP differently than the specialist functions responsible for trace gas oxidation in soil. Subsequent examination of the bacterial community structure was undertaken to disentangle the role of α- and β-diversity in explaining variations in the process rates.

Soil bacterial communities

There were more bacteria in the soil microcosms manipulated with dilution and antibiotic treatments than in the reference microcosm, based on the 16S rRNA gene copies (supplemental Table S1[1]). There was no evidence that variation in bacterial abundance caused by the diversity manipulation treatments was a significant factor

Fig. 1. Incidence of soil treatments on microbial processes. The boxplots displayed measured (A) CO oxidation rate, (B) H_2 oxidation rate, (C) CO_2 production, and (D) community-level physiological profile (CLPP) relative to reference soil microcosms. Microcosms are labeled with numbers representing soil dilution and the subscript "A" indicating addition of antibiotics. Treatments with the same lowercase letter are not significantly different by Dunn's test.

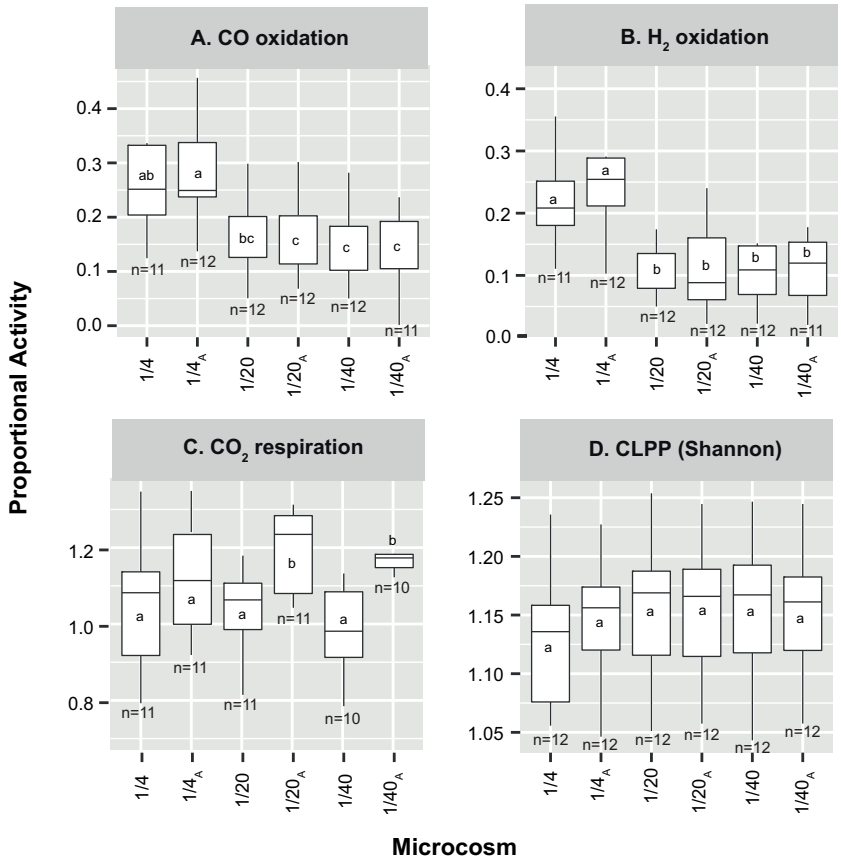


Table 1. Number of samples (n), sequencing effort, abundance of 16S rRNA gene, and α-diversity parameters of bacterial communities in soil microcosms.

Microcosm*	n	No. of sequencing reads	Species richness (q = 0)	Shannon's entropy index (q = 1)	Inverse Simpson's index (q = 2)
1/4	4	23 077 (12 210)	1476 (79)a	756 (75)a	334 (39)a
1/4_A	6	23 326 (6 624)	1423 (34)ab	678 (51)ab	266 (32)ab
1/20	7	25 526 (12 743)	1139 (37)bc	417 (29)abc	149 (18)bc
1/20_A	5	22 252 (13 890)	967 (90)d	292 (51)d	107 (17)cd
1/40	8	28 075 (12 725)	1029 (53)cde	380 (39)cde	149 (32)cde
1/40_A	2	25 095 (3 026)	981 (5)cde	306 (22)cde	111 (12)cde
Control	4	26 618 (17 799)	1378 (61)abc	830 (43)ab	476 (33)ab

Note: Average values are presented with standard deviation in parentheses. Different letters denote parameters discriminating treatments (Dunn's test).

*Microcosms are labeled with numbers representing soil dilution (1/4, 1/20, 1/40) and the extension "_A" indicating addition of antibiotics.

explaining differences in microbial processes noted in the previous section. Soil dilutions and antibiotic treatment were expected to exert an influence on bacterial species' richness and evenness. The partitioning of α-diversity into the three Hill numbers, q = 0 for species richness, q = 1 for the exponential of Shannon's entropy index, and q = 2 for the inverse of Simpson's concentration index, showed two distinct groups (Table 1). The first group comprised both microcosms of the 1/4 dilution and the 1/20 dilution without antibiotics in which neither species richness nor evenness was distinguishable from reference microcosms. The second group displaying a lower level of species richness and evenness than reference microcosms comprised the 1/20 dilution with antibiotics and both microcosms of the 1/40 dilution. These results indicated that the reduction of α-diversity of bacterial

Fig. 2. Incidence of soil treatments on bacterial community structure. (A) Relative contribution of dilution, antibiotic, and incubation (Block) in explaining variations in bacterial community structure (PERMANOVA, with asterisks denoting significant factors at $p < 0.01$). (B) Principal coordinates analysis (PCoA) of the bacterial community profiles represented by points, with different colors and shapes distinguishing treatments and replicates, respectively. (C) β-Dispersion of replicates within each dilution treatment (1/4, 1/20, and 1/40) and reference microcosms. Different letters above the boxes represent treatments for which β-dispersion is different by Dunn's test. (D) Unweighted pair group method with arithmetic mean dendrogram derived from the Bray–Curtis distance between each sample with different colors and shapes distinguishing dilution and antibiotic treatment, respectively.

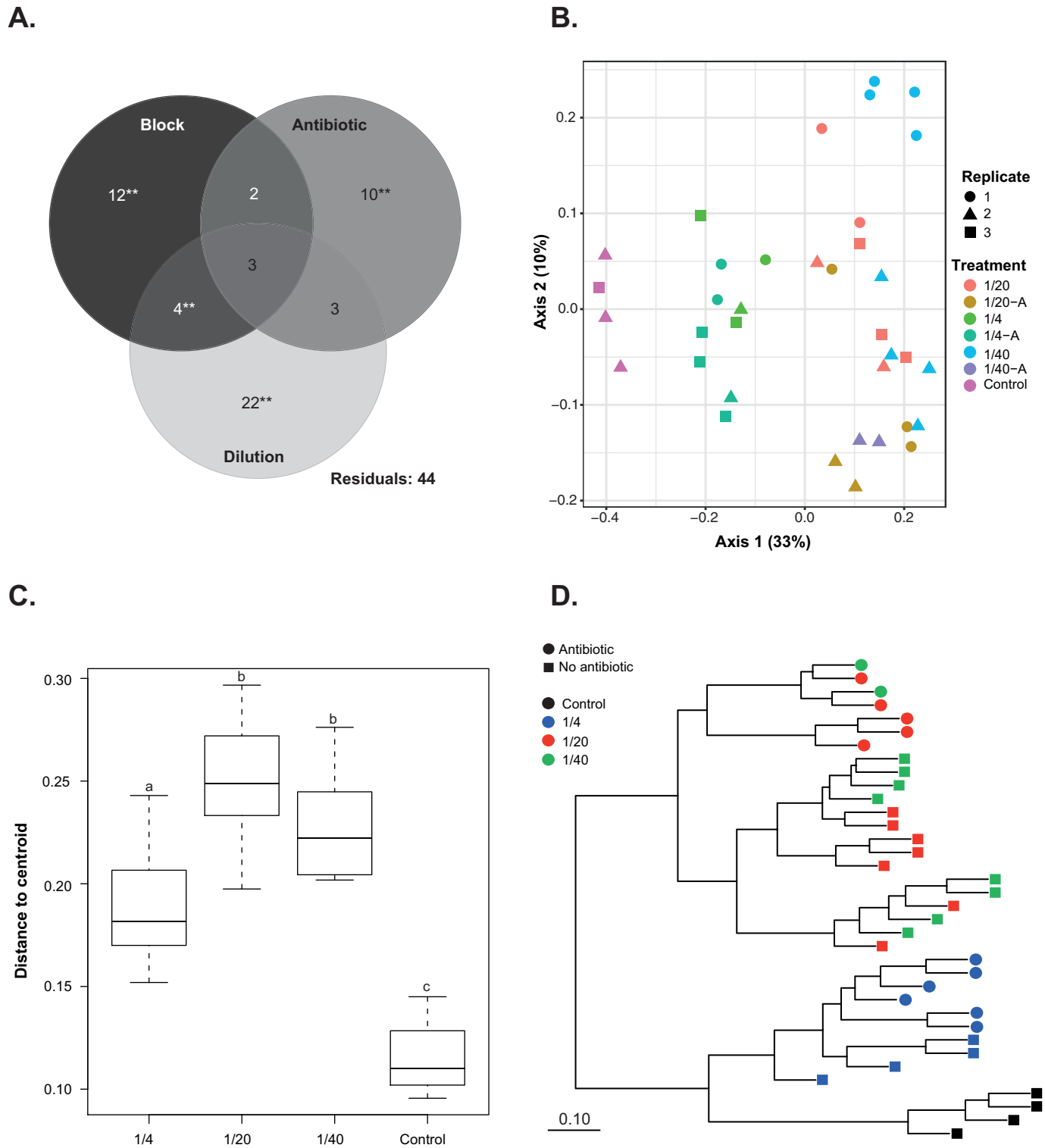
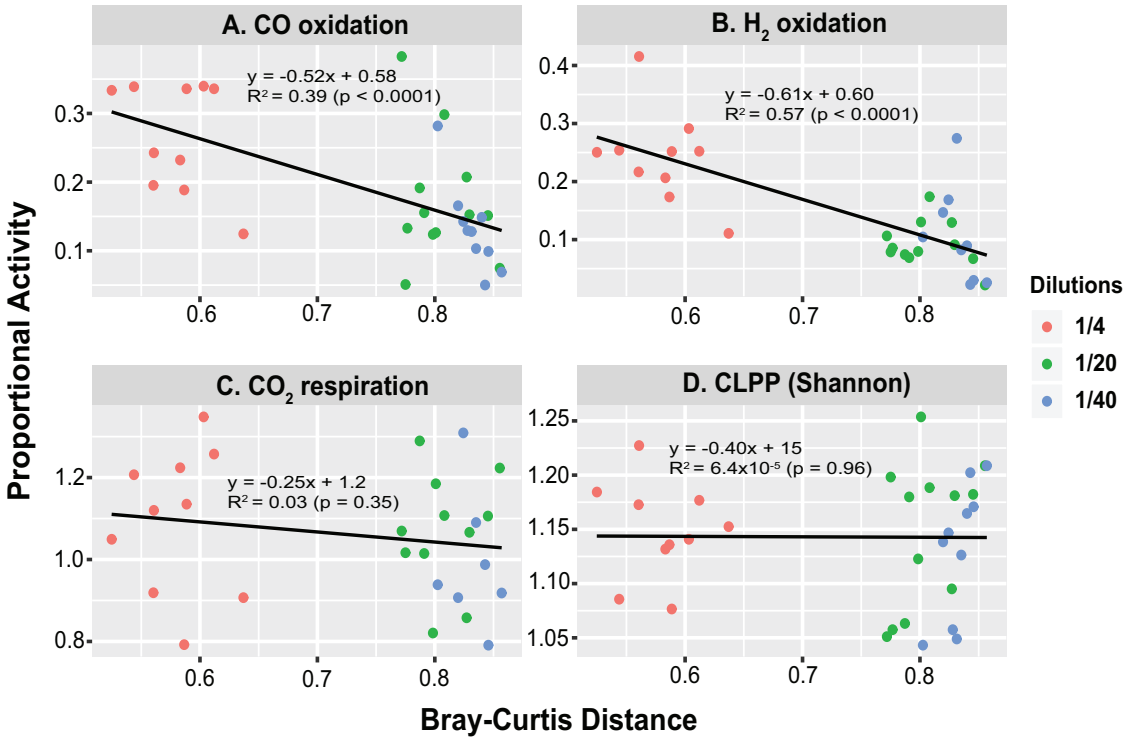


Fig. 3. Covariation in microbial processes and taxonomic composition in soil microcosms. Variation in (A) CO and (B) H_2 uptake, (C) CO_2 production, and (D) community-level physiological profile (CLPP) was explained by variation in bacterial community structure expressed as the Bray–Curtis distance from reference microcosms using linear regression.



communities could not account for the loss of CO and H_2 oxidation activity in the 1/4 dilution treatments.

Soil bacterial communities were dominated by *Proteobacteria* (41%), *Actinobacteria* (22%), *Bacteroidetes* (11%), and *Acidobacteria* (10%) (supplemental Fig. S1[1]). Diversity manipulation treatments exerted an influence on the composition of soil bacterial communities. The dilution treatment explained 22% of the variation in bacterial community profiles, while the block effect and antibiotics explained 12% and 9% of the variation, respectively (Fig. 2A). The impact of soil treatments was further supported by the ordination of the bacterial community profile in the reduced space of a PCoA explaining 43% of their divergence (Fig. 2B). In Fig. 2B, the dilution treatments explain the distribution of the microcosms along the first axis, while the second axis discriminates the three independent incubations (block effect). Distribution of the samples along the second axis displays an increasing dispersion as a function of dilution treatment. This observation was further supported by a β-dispersion analysis showing that variability of replicated profiles from the same treatment increases with the dilution level (Fig. 2C). Finally, the average Bray–Curtis distance separating each microcosm from the reference microcosms was computed (Fig. 2D) and used to generate models testing the covariation between the composition of bacterial communities and the process rates in soil.

Relationship between the composition of bacterial communities and the process rates in soil

The impact of microbial community manipulation (i.e., dilution and antibiotic treatments) on specialist and generalist functions was evaluated with single linear regression models (Fig. 3). Variation in CO and H_2 oxidation activities were explained by the model, with an increasing loss of activity as a function of intensification in alterations of microbial community profiles. In contrast, alterations in microbial community composition in soil explained neither variations in CO_2 production nor variations in CLPP. Comparison of the slope of H_2 and CO oxidation activity models suggests that HOB are slightly more sensitive than COB to shifts in diversity.

Discussion

Dilution to extinction is a meaningful approach to relate soil microbial diversity to biogeochemical process rates and their resistance to disturbance. Intuitively, dilution of multispecies microbial consortia before inoculation is assumed to remove rare species, which is arguably supported by the loss of species proportional to the dilution factor (Wertz et al. 2006). However, this assumption must be taken cautiously, since dilutions also exert an influence on dominant species for which proliferation is driven by interactions with other members of the communities and compounds transferred with the inoculum (Howard et al. 2017). Alteration of dominating species points to evenness as another relevant compo-

nent driving the biodiversity–ecosystem functioning relationship. Indeed, evenness of microbial communities promotes the resistance of processes to disturbance, especially in the case of selective pressure compromising the fitness of the few dominating representatives of the functional guild (Wittebolle et al. 2009). In this study, a combination of moderate dilution and antibiotic treatments was selected to reduce the massive loss of rare species, while altering evenness of the communities. Moderate diversity impairment was investigated owing to the importance of this phenomenon in different anthropic disturbances of soil microbial diversity, such as land-use change, which have been shown to exert a more significant effect on the microbial community structure than on species richness (Carvalho et al. 2016; Crowther et al. 2014; da C Jesus et al. 2009). Indistinguishable species richness and evenness between treatments and reference microcosms supported the relevance of moderate dilution treatments.

Both generalist functions involved in carbon cycling were slightly stimulated, while oxidation of CO and H_2 specialist functions decreased in response to changes in microbial community structure. The exact mechanism explaining enhanced CO_2 production in the 1/20 and 1/40 dilution microcosms amended with antibiotics is unknown, but similar observations were reported in soil amended with tetracycline, ciprofloxacin, sulfamonomethoxine, and combined applications (Ma et al. 2014). Dilution and antibiotic treatments promoted diversification of CLPP in soil. Similar results were obtained in soil microcosms supplemented with chlortetracycline (Liu et al. 2015). Processes that triggered alteration in CO_2 production and CLPP in response to soil microcosm treatments are multiple, encompassing sensitivity of microbial interactions controlling carbon turnover to diversity and antibiotics (Zhang and Zhang 2016), as well as a priming effect caused by the denaturation of organic matter during soil sterilisation and the lysis of sensitive cells caused by the supplemented antibiotics (Vaclavik et al. 2004). As expected, both generalist functions were nonsensitive to modification in β-diversity, as explained by the high degree of redundancy of these functions in soil microorganisms. Indeed, the sensitivity of microbial processes mediating carbon turnover depends on substrate recalcitrance, with mineralization of labile plant residues more resistant to diversity loss than mineralization of recalcitrant organic matter in soil (Baumann et al. 2013; Maron et al. 2018).

Recovery of CO and H_2 oxidation activities in the 1/4 dilution treatment displaying neither loss of species richness nor evenness was 30% in comparison with reference microcosms. Interestingly, this loss of activity in parallel with the alteration in the taxonomic composition of the soil microbial community was of the same magnitude as the range of activity observed in response to environmental cues, including changes in land-use or soil physicochemical properties. For instance, measurements of H_2 and CO soil uptake rates along a chronosequence encompassing vegetation colonisation of soil volcanic deposits showed that a barren site displayed 19% and 35% of the activities measured at the end of the succession site, respectively (King et al. 2008). The impact of land-use change also was observed in a deciduous forest converted to maize and larch monocultures, displaying 80% and 88% lower potential CO oxidation activity, respectively (Quiza et al. 2014). It is currently assumed that the response of H_2 and CO oxidation activities to land-use change is triggered by modification of soil organic content that correlates with activities (Conrad and Seiler 1985), but soil microbial diversity has never been considered in past investigations. In addition to land-use, pH and substrate-induced respiration explain the variation in H_2 oxidation activity measured in soil (Gödde et al. 2000). According to these previous models, soil displaying a pH of 6 is expected to show 22% of the H_2 oxidation activity measured in a neutral pH soil, if the soil water holding capacity is 30% and both soils display the same substrate-induced respiration rate. These comparisons highlight the need to disentangle the relative contribution of soil abiotic features and microbial diversity in explaining H_2 and CO soil uptake activities. Bridging this knowledge gap could improve the resolution of temporal and spatial distribution models of atmospheric H_2 and CO concentrations into which H_2 and CO fluxes are parameterized with variables encompassing temperature, primary productivity, and land-use types.

Based on our process rate measurements conducted in soil microcosms, we conclude that composition instead of richness of microbial communities drives high-affinity CO and H_2 oxidation activities in soil. Identification of potential species involved in H_2 and CO oxidation activities in each treatment was not attempted due to the incongruence between classifications of HOB and COB in functional gene-based (NiFe-hydrogenase and CO-dehydrogenase) and 16S rRNA gene-based (Constant et al. 2011; Quiza et al. 2014) phylogenies. The observed loss of oxidation activities begs the question of whether the composition of COB and HOB functional guilds, their mixotrophic lifestyle, or their interactions with other members of the community are drivers of H_2 and CO soil uptake activities. Addressing this question is an exciting challenge requiring innovative strategies that fall beyond conventional molecular surveys. Indeed, loamy sand soils were shown to harbor complex communities of COB and HOB represented by hundreds of genotypes (Khdhiri et al. 2017; Lalonde and Constant 2016). Partitioning the contribution of individual genotypes to community-level process rates is hampered by the lack of information regarding cell-specific activities of each member of the communities. Furthermore, compelling experimental evidence supports the importance of

microbe–microbe interactions in determining whole community process rates. The mechanisms behind such interactions are far from being elucidated, but the exchange of signaling volatile organic compound molecules was proposed to explain stimulation of CH_4 oxidation activity of methanotrophic bacteria cultivated in the presence of heterotrophic bacteria unable to scavenge methane (Ho et al. 2014; Veraart et al. 2018). These interactions are further complicated owing to the mixotrophic lifestyle of microorganisms using trace gas. In the case of CO and H_2 present at the trace level, their oxidation was shown to supply survival energy requirement under starvation (Liot and Constant 2016) or growth requirement in combination with other substrates (Greening et al. 2014). The use of synthetic communities is seen as a promising avenue to complement the effort of diversity erosion experiments aimed at determining the relationship between microbial diversity and functioning in soil.

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