Reproducible Propagation of Species-Rich Soil Microbiomes Suggests Robust Underlying Deterministic Principles of Community Formation

Senka Čaušević¹, Janko Tackmann², Vladimir Sentchilo¹, Christian von Mering² and Jan Roelof van der Meer¹

1) Department of Fundamental Microbiology

University of Lausanne

1015 Lausanne, Switzerland

2) Department of Molecular Life Sciences,

University of Zürich

8006 Zürich, Switzerland

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ABSTRACT (max 150 words)

Microbiomes are typically characterised by high species diversity but it is poorly understood how such system-level complexity can be generated and propagated. Here, we used soils as a relevant model to study microbiome development. Despite inherent stochastic variation in manipulating species-rich communities, both laboratory-mixed medium complexity (21 soil bacterial isolates in equal proportions) and high-diversity natural top-soil communities followed highly reproducible succession paths, maintaining distinct soil microbiome signatures. Development trajectories and compositional states were different for communities propagated in soils than in liquid suspension. Microbiome states were maintained over multiple renewed growth cycles but could be diverged by short-term pollutant exposure. The different but robust trajectories demonstrated that deterministic taxonomic characteristics underlie reproducible development and self-organized complexity of soil microbiomes within their environmental boundary conditions. Our findings also have direct implications for potential strategies to achieve controlled restoration of desertified land.

TEASER (125 characters)

Species-rich soil microbiomes grow and propagate reproducibly despite inherent stochastic complexity, paving the way for soil restoration.

INTRODUCTION

Microbial communities are highly complex systems that self-organize seemingly spontaneously within the spatiotemporal, physical, chemical and biological boundary conditions of their environment or their host. The living microbial systems within these boundaries (the ‘microbiomes’) have attracted recent wide interest, due to their crucial contributions to ecological and biosphere processes (1-3), as well as to plant (4), human (5) and animal health (6). However, despite their widely recognized importance, there is still a large gap in understanding the general principles underlying microbiome

development and functioning, as well as their amenability for functional and compositional engineering.

To a large part, our current understanding of the operating principles of microbiome formation comes from bottom-up studies with limited species numbers in synthetic ecosystems (7-10). Interspecific interactions are assumed to be the generators of community self-assembly and of emerging system-level metabolic properties (11, 12). For example, range expansion experiments with 2–3 strains have demonstrated the quality, types and importance of interspecific metabolic interactions and spatial structuring (13-18). To some extent, higher-order community composition can also be successfully predicted from empirical measurements of paired growth interactions (10, 19). However, multi-species interactions can give rise to feedback mechanisms that provide reciprocal control on their growth (10), or lead to multistable paths as a consequence of individual growth variation (20). Interspecific interactions further emerge in dependency of initial growth conditions and environments (21, 22), and with increasing species complexity, non-additive effects may arise (23). The emergence of interspecific interactions depends on the spatial distance between cells (24) and, consequently, may be different in highly fractured environments such as soil, as opposed to liquid suspension (25-28). The question is thus whether developmental paths of species-rich communities are inherently stochastic and, in that sense, mostly irreproducible, or whether their taxa-composition provides robust self-organizing properties that will only diverge as a result of differences in environmental boundary conditions. In order to test this question, it is important to design studies that can bridge from the very simplified synthetic communities alluded to above, to more realistic species-diverse communities.

The major aims of the underlying work were thus twofold: first, to develop a tractable system to generate and propagate species-rich communities, and secondly, to study their developmental paths and resulting compositional states under different environmental boundary conditions and culturing

regimes. We specifically focus on soil microbiomes, which comprise among the most diverse known microbial communities with up to 50 000 species (29) and 10^{10} cells per gram of material (30). The soil microbiome is of crucial importance for soil fertility and plant growth, for water purification and biogeochemical cycles (1, 31, 32). Soils are threatened world-wide as a result of land management, agricultural practices, erosion, waste deposition or chemical spills, leading to a general loss of soil structure and diversity (33, 34). Soil microbiomes are thus highly relevant and one of the options for restoration of perturbed communities is through rational management, although current methods, e.g., soil transplantation or inoculation are very much a black box (35-38).

To obtain a realistic culturing system to propagate species-rich soil communities, we used autoclaved natural soil matrix replenished with soil carbon and nutrient extract. We contrast two types of soil communities, one composed of 21 indigenous soil isolates covering four major phyla (called *synthetic community* or SynCom), and the other comprising a species-rich soil microbial mixture directly washed and purified from top soil (NatCom, for natural community). Both communities were inoculated at low density in the reconstituted soils to allow growth and colonization, under two different culturing regimes. The first consisted of a single long-term incubated batch sampled after one week, two and six months, to favor slow-growing bacteria. The other consisted of multiple dilution-growth cycles of one week each, to favor community stabilization and test resilience to chemical perturbation. Community trajectories in soils were further compared to that in liquid suspension. Compositional changes were inferred from 16S rRNA gene amplicon sequencing and community signatures were compared to all available world-wide characterized soil and rhizosphere communities. Our results indicate highly reproducible species-rich community development for both synthetic and natural soil inocula. Developmental trajectories depend on incubation regimes and environmental conditions, suggesting robust deterministic self-organizing principles.

RESULTS

Design of controllable soil microbiome culturing systems. Standardized culturing systems for studying the development and succession of species-rich microbial communities were produced from sterile nutrient-complemented soils that could be inoculated, grown and diluted into fresh material, as is common for typical liquid culturing (Fig. 1A). The soil matrix was obtained from a riverbank sediment, twice autoclaved and complemented with a sterile soil nutrient extract from a topsoil (SE, soil extract). SE-reconstituted soils maintained general properties of natural soil. The final matrix of autoclaved soil + SE had a slightly basic pH, which was indifferent from the starting material (Fig. 1B). Autoclaving reduced Mg-levels, but did not significantly change Ca, P and Fe levels (Fig. 1C). UV/Vis fluorescence measurements indicated organics-rich material in SE (Fig. 1D, Supplementary table S1), which retained broadly the same six groups of humics and fulvics (39) as in the original soil (Fig. 1E, Supplementary table S2, $p = 0.3078$ Fisher's exact test of relative abundances). Reconstituted soil showed increased availability of lower molecular weight compounds due to SE addition, as evident from the increased absorbance ratio at 250 nm to 365 nm (by 0.73 and 0.24 respectively; Fig. 1D, Supplementary table S1). Organic matter analysis in the SE-supplemented soils indicated an average of 1.5 mg total organic carbon g^{-1} soil matrix and 0.3 mg total N g^{-1} (Supplementary table S3). Assuming carbon needs of 200 fg C per cell and a g-C g-C $^{-1}$ yield ratio of 20%, this would permit the development of a community of roughly 10^9 cells g^{-1} .

Generation and propagation of species-rich soil microbial communities. Autoclaved soil+SE was inoculated with starting community suspensions at 10^7 cells ml^{-1} , producing an equivalent of 10^6 cells g^{-1} soil at the set 10% gravimetric water content. Community inocula consisted either of a washed and purified microbial cell suspension from top-soil (NatCom) or a suspension of 21 soil bacterial isolates mixed in equal relative abundances (SynCom, Table 1). Inoculated soil microcosms with NatCom suspensions after one week reached $2.8 \pm 2.4 \times 10^8$ cells g^{-1} (one SD, $n = 4$, Fig. 2A), an estimated 280-fold increase from the inoculum size (~ 8 doublings). Averaged across all 1-week culturing cycles, the NatComs maintained at $4.7 \pm 1.1 \times 10^8$ cells g^{-1} soil. This was an average of 3.5 times higher than the

community size obtained in (liquid) SE alone (calculated on a per ml-basis, Fig. 2B, $p = 0.0004$, one-tailed t-test). This suggested that all easily accessible carbon was utilized during each week of incubation time and that communities reached semi-stationary phase (see below) before they were transferred to fresh soil medium. There was no discernable trend in the NatCom cell numbers as a function of growth cycle (Fig. 2A, NatCom linear regression: 0.0211, $p=0.4371$ compared to slope = 0). SynCom inoculum mixtures (Table 1) increased from 1×10^6 cells g^{-1} soil to a stable average density after every growth cycle of $1.11 \pm 0.32 \times 10^9$ cells g^{-1} soil, which was 2.4 times higher than that of the NatCom (Fig. 2A, $p = 9.9 \times 10^{-9}$ unpaired two-sided t-test, $n = 34$). In comparison to its liquid SE suspension, the average one-week SynCom density in soil + SE was four times higher, similar as for the NatCom (Fig. 2B, $p = 0.0002$, one-tailed t-test).

In contrast to the communities propagated under the 1-week growth/dilution cycles, those maintained under a single long incubation after initial growth in the first week, decreased in size as inferred from community DNA yields (Fig. 2C). NatCom DNA yields in soils decreased by 2–4 fold after 2 and 6 months, but not in liquid SE (Fig. 2C, $p = 0.0101$). SynCom sizes declined by 3– and 6-fold after 2 and 6 months, respectively, both in soils and liquid (Fig. 2C). This decrease may have been due to carbon limitation, consequent cell death and carbon turnover. Overall, these experiments indicated that high-density complex communities developed in both regimes and persisted over long times.

Compositional state trajectories during culturing. The NatCom compositional dynamics under the two growth regimes was assessed from changes in the relative taxa abundances, determined by 16S rRNA gene amplicon sequencing using 99% identity thresholds for OTU assignment. The mean detected richness reduced from 233 in the inoculum to 22 (9%) after the first week, which slowly increased to 37 (16%) after the 8th incubation cycle (Fig. 3A). In addition, 75% of the taxa after the 1st cycle (week 1) were not detected in the inoculum (Fig. 3A, magenta bars), suggesting that community succession was initially driven by rapidly growing low abundant taxa. Non-metric multidimensional scaling (NMDS) analysis confirmed the strong deviation of both soil + SE and liquid SE microcosms

from that of the original inoculum (Fig. 3B). Growth cycles resulted in closely clustering communities (Fig. 3B, T1–T8), whereas the single long incubations showed succession and higher similarity to the inoculum state (Fig. 3B, 2 and 6 months). NatCom development in soil + SE was distinct from that in liquid SE alone, indicating that the soil environment (and resulting pH differences) may have driven the community differentiation (Fig. 3B, adonis $p = 0.001$ with beta-dispersion of $p = 4.38 \times 10^{-10}$). Although replicates clustered coherently in NMDS (Fig. 3B), there were obvious stochastic effects of compositional succession, illustrated by variation in appearance and relative abundance of individual taxa among replicate inoculations after the first week of incubation (Fig. 3C, e.g., Rhizobiales, Sphingomonadales and Enterobacteriales). Replicate variability was higher at OTU level than at order level (Fig. 3D, Supplementary figure S1), suggesting conserved functional order traits that permit strains from such groups to quickly colonize new environmental niches. NatCom replicates kept a relatively strong individual signature independent of multiple growth/dilution cycles (most evident with the “–2” replicate, Fig. 3E), which mostly converged in long-term incubations (Fig. 3E, L6 samples). This might be due to initial stochastic compositional variations that influence growth in the first incubation and from there on, propagate the states of regrown communities. Mathematical simulations of community growth and composition suggested that this variation may be due to subsampling effects of rare taxa with high growth rates within a finite-sized inoculum (Supplementary figure S2). Initially composed of 18 phyla, only five were detected in NatCom replicates after the first growth cycle, and four more appeared after cycle 8 (Fig. 3F), indicating that their members were present but undetectable at our sequencing depth. In contrast, long-term incubated NatCom showed members of ten phyla, indicating that this growth regime permitted higher diversity, perhaps by avoiding bottlenecks of the dilution/growth cycles on slow-growing members (Fig. 3F). This showed that species-rich soil communities can be grown and maintained with relatively constant composition over multiple dilution cycles, despite having inter-replicate stochastic strain variability. Culturing in SE-reconstituted soil clearly provided additional benefits to the community, since both its size (Fig.

2B) and its richness remained larger (by 12.02% with growth cycles and 9.31% in the long batch regime, Supplementary figure S3) than that in SE liquid suspension.

Development of medium complexity synthetic soil microbiome recapitulates natural states. To corroborate the observed succession and development patterns in NatCom, we followed changes in the defined SynCom. The SynCom was selected from a total of 172 recovered pure cultures based on different colony morphologies and growth characteristics grouped into 52 different genera belonging to four phyla (Supplementary table S4). Except for the phylum Verrucomicrobia, these isolates covered the major phyla observed after the first NatCom growth cycle (Actinobacteria, Bacteroidetes, Firmicutes, and Proteobacteria, Fig. 3F), with some redundancies (Table 1, Fig. 4A).

In contrast to NatCom, the compositions of the SynCom showed clearer succession during the first three growth/dilution cycles, after which they stabilized. This was evident from a loss of apparent diversity (i.e., within the sequencing threshold for community membership), from 21 to 9–10 detectable members after the fourth cycle (Fig. 4B), and a sharp decrease of Shannon index (Fig. 4C). The T_0 –sample (taken 30 min after inoculation into the soil) resembled the inoculum closely (Bray-Curtis distances of 0.26 ± 0.02 , while the distance between inoculum and T8 was 0.65 ± 0.03), showing minimal bias introduced by cell extraction (Fig. 4D). Initially higher relative abundances of *Pseudomonas* strain1 and *Rahnella* during the first-to-third growth cycles were replaced by *Pseudomonas* strain 2, *Lysobacter*, *Variovorax*, and *Caulobacter* as the dominant members. Finally, also *Cohnella*, *Rahnella* and *Tardiphaga* regained sizeable proportions of the SynCom (Fig. 4D). Independent SynCom replicates followed highly similar developmental paths (Fig. 4E), in terms compositional changes, loss of diversity and reaching semi-stable compositions after the 4th cycle (Fig. 4B-D). SynCom replicates clustered coherently over time and did not maintain individual replicate signatures as NatCom (Supplementary figure S4). SynCom compositions in soil + SE differed significantly from that of the inoculum and those grown in liquid SE suspension (Supplementary figure S5; adonis $p = 0.001$; betadisper $p = 0.0002$). Similar as for the NatCom, the long incubation regime

led to higher detectable diversity of 18-20 strains from the initial 21 after 2 and 6 months (Fig. 4B, C, and E, $p = 0.001$ from adonis and $p = 0.0002$ for beta-dispersion). This included higher relative abundances of *Mesorhizobium*, *Luteibacter* and *Devosia* compared to e.g., *Pseudomonas* (Supplementary figure S5).

SynCom and NatCom retain soil signatures but differ in replicate variability. In comparison to a wide set of available soil communities ($n = 110,928$), both SynCom and NatCom compositions grown in soil kept clear soil community signatures (Fig. 5A). Interestingly, SynCom compositions located closer to ‘plant rhizosphere’ communities, possibly due to the culturing isolation bias (Fig. 5A). NatCom grouped closer to ‘field soils’, whereas the inoculum, as expected, had a ‘forest’ soil signature (Fig. 5A). There is not a clear single factor underlying this environmental signature, although soil-pH (as far as present in the meta-data) seems an important variable (Fig. 5B). Both SynCom and NatCom became largely dominated by Alpha- and Gammaproteobacteria, but were notably different in the relative abundances of Bacteroidetes (contributing 30-50% in the NatCom) and Firmicutes (5-10% in the SynCom) (Fig. 5C). SynCom replicate variability was twice as low as that of the NatCom (Fig. 6D, $F=17.495$, $p=5.19 \times 10^{-5}$, ANOVA), with high replicate homogeneity (i.e., the replicate Bray-Curtis distance from the community centroids, ranging from 0.01 to 0.20; Fig. 5D). The reason for this is likely the lower number of starting strains in the SynCom and lower likelihood of stochastic variations as a result of subsampling upon dilution (as in, e.g., Supplementary figure S2).

Chemical perturbation changes SynCom trajectories. In order to investigate the stability of developed communities, we tested their resilience towards the moderate toxic compound toluene, as an example of recurrent soil pollution with organic solvents (40). To this end, we split the stabilized ten SynCom replicates in two groups of five after the fifth growth cycle; one series of which was exposed to toluene vapor during the next one-week cycle, the other cultured as before. After this exposure period, all SynCom replicates were diluted again into sterile, non-polluted SE-amended soil; and

growth cycles were continued as before. Exposure to toluene significantly lowered the attained community sizes (Fig. 6A, $p = 0.0231$, one-tailed t-test on all replicate samples and time points, $n = 15$). In contrast, toluene exposure did not lead to significant changes in richness (Fig. 6B, $p = 0.4235$, two-way ANOVA), nor did it influence Shannon diversity (Fig. 6C, $p = 0.2128$, two-way ANOVA). Varying effects were observed on individual SynCom members, which either slightly (Fig. 6D, e.g., *Variovorax*, 25.8% decrease), or drastically decreased in population size (Fig. 6D, 99.9% decrease of *Burkholderia*, see *Devosia* and *Flavobacterium* in Supplementary figure S6), whereas some increased in abundance (Fig. 6D, *Microbacterium*, *Cohnella*). Inter-replicate variability was not significantly affected with toluene exposure, even during the first week of recovery (Fig. 6E, $F=0.8973$, $p=0.4994$, ANOVA). Community signatures in exposed SynCom remained distinct from those of the non-exposed communities even after the 8th cycle (Fig. 6F, adonis $p = 0.001$; betadisper $p=0.1024$). Altogether, this indicated that stable soil communities can be perturbed by chemical (toluene) exposure, which changes their compositional trajectories in a long-lasting manner.

DISCUSSION

We showed reproducible assembly, succession and composition of both a high-complexity NatCom (starting from washed mixed soil inoculum, containing 18 bacterial phyla), as well as a medium-complexity SynCom with 21 culturable strains (covering four major bacteria phyla) in a soil culturing system that enables soil-to-soil transfers. The standardized soils were generated from riverbank sediment supplemented with a soil extract, which provided realistic available carbon and nitrogen compounds for growth of community cell densities similar to what is typically found in top soils (30). Both NatCom and SynCom retained typical soil and plant rhizosphere microbiome signatures, and thereby represent excellent test beds for plant growth, community management or soil resilience studies that require complex and reproducible starting communities. Both growth regimes, either imposed as multiple one-week growth and dilution cycles in soil, or as single batch long incubation

(up to 6 months) favored establishment of high species diversity, which in short incubations (one week) was dominated by relatively fast-growing opportunistic strains. Cultured SynCom on average had higher cell densities on the same substrate than NatCom, which might be due to their inoculum being exclusively of bacterial origin, without any potential phage or protist predators that might have been present in the washed mixed microbial top-soil NatCom inoculum mixture. The sizes of both communities, however, moderately decreased over long incubation periods (2 and 6 months), suggesting some cell death and consequent carbon turnover. The long-time incubation may have allowed growth of members from slow-growing phyla that are difficult to obtain in pure culture (e.g., Acidobacteria, Gemmatimonadetes).

One of the key surprises of our work is the demonstration of highly reproducible trajectories and compositional states of medium-to-high species-diverse soil communities. Low variability among starting replicate soil communities makes it easier to detect effects of inoculant bacteria or fungi in relation with plants for improving plant health (41), to investigate the influence of bioaugmentation agents in pollution removal (35, 36), or to address fundamental ecological questions on community resilience (42). These growth properties of SynCom and NatCom can be fruitfully exploited in future work to study fundamental ecological questions of species redundancy, resilience, or invasion resistance (42, 43). The high reproducibility of community development under realistic culturing (e.g., soil) was counterintuitive. Considering the complexity of the provided nutrients, highly fractured soil environment and species-diverse inocula, we expected that stochastic small variations in experimental manipulations would lead to chaotic system behaviour. Contrary to this intuition, the medium complexity (21-member) SynCom developed highly reproducibly among ten replicates, with similar succession patterns, total community sizes and relative species abundances. Initially (inoculated) balanced species proportions were quickly replaced by coherent compositional trajectories and states during multiple growth/dilution cycles. The attained SynCom compositional states were dependent on the type of incubation regimes (growth cycles versus one-batch long time),

and specific environments (soil+SE or liquid SE) but retained no individual replicate signatures. Compositional states could be perturbed by short-term chemical exposure, but then reproducibly continued on slightly different trajectories. In contrast, NatCom compositions showed more stochastic variability among replicates, and individual replicate signatures were retained to some extent in the growth/dilution cycles. Community growth simulations suggested that the reason for increased stochastic replicate variability may lie in population bottlenecks arising from finite-sampling of high diverse community inocula with rapidly growing colonizers, leading to a state which then self-propagates in subsequent growth/dilution cycles. Despite this, NatCom replicate variability collapsed at a higher phylogenetic level, suggesting similar functional and redundant properties in the complex starting inoculum that are selected during colonization of pristine growth environments. Long-time incubations also dissipated NatCom compositional variations to a large extent. Both SynCom and NatCom in soil microcosms developed and maintained clear soil community signatures. This indicates strong deterministic influences of the initial species composition on the community development trajectories (i.e., self-organizing complexity) within its system boundaries and the prevailing environmental conditions.

Several authors have reiterated that the origins of microbiome complexity remain fundamentally unknown (23, 44) and that general rules governing community assembly and functioning are difficult to deduce (21, 45-47). It is clear that community growth and development are influenced by a myriad of factors such as growth substrates, spatial structures, and presence of other chemical compounds (44, 48). The more complex carbon substrates deployed in this study possibly require and facilitate a wider range of metabolic capacities and therefore maintained higher functional diversity (30–40 OTUs in NatComs), than in previous experiments starting with soil and phyllosphere communities but grown on a single carbon substrate (5–12 exact sequence variants) (49). Community development is further expected to be dependent on emerging interspecific interactions leading to transcending systems-level functionalities (21, 45). Indeed, both NatCom and SynCom development seemed strongly

determined by their starting taxa compositions, on top of which the environmental boundary conditions (i.e., soil versus liquid) influencing the community trajectories. The difference in compositional trajectories and states in soil and liquid, despite containing the same complex nutrient resource availability (soil extract), may be due to different types or magnitudes of interspecific interactions arising in the spatially structured, disconnected and heterogenous growth environment of the soil as opposed to the liquid-suspended growth (25). Soils are expected to provide unique ecological niches (1, 50), and their aggregates affect nutrient availability and gradients in electron donors and acceptors (26-28, 48, 51). Indeed, SynComs and NatComs maintained on average higher species diversity in soil microcosms than equivalent liquid cultures, suggesting emerging favorable dependencies, which permitted more phyla to sustain and grow (25). Suggestive for this is that members belonging to the Acidobacteria, Verrucomicrobia and Planctomycetes proliferated in all NatCom microcosms, whereas we did not manage to culture them individually using the same nutrient substrates.

Natural soil communities probably only very rarely have the opportunity to colonize a pristine soil environment, except perhaps for soil transplants or soil construction work, grubbing, glacier retreats or other (52, 53). At a large scale (cm – m), the composition of complex natural soil communities is stable, but may undergo temporal and very local fluctuations driven by nutrient gradients from plant roots, burrowing fauna, rainfall, seasonal temperature changes or other (54-56). In that sense, our long-term incubation regimes resembled new soil colonization events, eventually leading to a mature state composition, typically comprised of several abundant members and a vast fraction of extremely low abundant species (“rare biosphere”) (57-59). The regime of imposed growth cycles may reflect what happens at sudden bursts of newly available carbon in the soil. As the NatCom experiments demonstrated, some “rare taxa” in the mature compositional state as isolated from the natural soil (Gammaproteobacteria, known generalists) rapidly proliferated in the first week of incubation, with Alphaproteobacteria and other phyla appearing only later, as has been observed before in natural

systems (47, 59). Some rare taxa may thus rather represent “conditionally rare taxa”; those with radically changing abundances depending on space and nutrient availability (60). The specific roles or capacities of those taxa to become more abundant over time remain unclear for now, and could be due to factors such as use of different (refractory) carbon substrates, predatory lifestyles, different nutrient requirements or forms of metabolic dormancy to remain viable for longer. From an engineering perspective the maintenance of temporary community compositional steady states by the cycling growth-dilution regime is interesting and suggests an avenue for approaches that aim to keep relatively constant species proportions in mixed communities over time. Reproducible propagation of soil communities will also be key for restoration efforts on degraded or desertified land that aim to bring back healthy soil life.

MATERIALS AND METHODS

Preparation of a natural soil community. A natural mixed microbial community (NatCom) was washed from batches of 20 g taken with a sterile metal spoon from the 5 cm topsoil layer after removal of twigs, roots and leaves (Dorigny forest, University of Lausanne, 46°31'16.4"N 6°34'43.0"E). Soil batches were immediately transported to the lab and processed within 1 h. The soil was sieved through a 3-mm mesh to remove large particles. Microbial cells were detached from soil particles by mixing with sterile 0.2% (w/v) tetrasodium-pyrophosphate decahydrate solution (pH 7.5, Sigma-Aldrich), and then purified by sucrose gradient solution centrifugation as described by (61). The cell suspension recovered after sucrose gradient centrifugation was twice washed with sterile saline solution (0.9% NaCl) and resuspended in the same. Serial dilutions were stained with SYBR Green I and cell numbers were counted using flow cytometry (see below). For inoculation into microcosms, the cell suspension was diluted in soil extract (SE, see below) to 10^7 cells ml⁻¹. Subsamples of the NatCom suspension were used for DNA extraction and 16S rRNA gene amplicon sequencing (see below).

Preparation of the synthetic soil community. Individual soil isolates were obtained from similar NatCom suspensions of the same soil location, additionally purified using Nycodenz gradient (61), diluted and plated on different media, as suggested by Balkwill and Ghiorse (62). We used PTYG medium (containing, per L: 0.5 g glucose, 0.5 g yeast extract, 0.25 g peptone, 0.25 g trypticase, 0.6 g $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.07 g $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 15 g agar), or soil extract medium (see below) solidified with 1.5% agar (Agar bacteriological, Difco), either at pH 4.5 (adjusted with hydrochloric acid) or at pH 7.5 (with sodium hydroxide). All plates were incubated at room temperature (23 °C) for 2 weeks. In total, 172 morphologically distinguishable colonies were selected, purified to homogeneity by streaking on the same medium, regrown in PTYG and stored in 15% (v/v) glycerol at –80°C. Strains were identified and taxonomically positioned by full length 16S rRNA gene sequencing (see below).

A set of 21 isolates representing different major phylogenetic and culturable groups (Table 1) were selected to assemble a synthetic soil community (SynCom). To prepare the SynCom inoculum, individual strains were plated from –80° stocks on PTYG agar and grown for 4 days at room temperature. Cells were then collected from the plates by washing with 5 ml of soil buffer (containing per L, 0.6 g of $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.1 g of CaCl_2 and 1.8 ml of 5 x M9 minimal salts solution [BD Biosciences]). Individual cell suspensions were serially diluted in soil buffer and stained with SYBR Green I for 15 min in the dark, according to manufacturer’s instructions (Invitrogen), after which cell numbers were counted by flow cytometry (see below). Pure cultures were then diluted in soil extract (SE, see below) and mixed to obtain a suspension of in total 10^7 cells per ml, and with approximate equal abundances of each individual member.

Soil microcosm preparation. Both NatComs and SynComs were cultured and passaged in semi-natural sterile soil systems, based on a coarse silt supplemented with a sterile soil extract solution. The soil matrix was prepared from riverbank sediment (0-10 cm horizon) of the Sorge river sampled at the campus of the University of Lausanne (46°31'22.4"N 6°34'31.7"E). The material was transported to

the laboratory, spread in 5 cm layer in trays and air-dried in a ventilated hood at 23°C for two weeks, followed by double sieving to retrieve the 0.5-3 mm sized soil fraction. Sieved soil was divided in 2 kg portions, autoclaved for 1 h at 120°C and dried for an additional seven days as described above. Batches of soil (90 g for the first inoculation series, 80 g for subsequent transfers) were then distributed into 500-ml Schott borosilicate glass flasks with plastic screw cap and seal. Individual flasks with soil were again autoclaved (20 min, 120°C) to kill any remaining spores and vegetative cells. The sterility of the soil was confirmed after the second autoclaving by sampling batches of 10 g with a sterile glass spoon, mixing with 20 ml sterile 0.2% pyrophosphate solution and vortexing for 1 min at maximum speed (Vortex-Genie 2, Scientific Industries, Inc.), after which aliquots of 100 µL were plated on three different agar media: PTYG (see above), R2A (DSMZ GmbH) and Nutrient Agar (BD Biosciences). Absence of grown colonies after 3 weeks incubation at room temperature was taken as indication for the material to be sterile. All microcosms used in the study originated from the same batch of sieved soil.

As source of nutrients for all microcosms we produced a soil extract (SE) from the same soil as used for the NatCom and the SynCom isolates (see above), as follows. Top soil material (1–5 cm layer, 6 kg) was sampled as before and mixed in a 1:1 volumetric ratio with tap water in batches of 2 kg. The mixture was autoclaved (1 h, 120°C) mixed and left to settle overnight. The resulting supernatant was decanted into sterile 250 ml centrifuge tubes, centrifuged at 5000 × *g* for 15 min to remove solids and pooled into 500 ml Schott flasks. This solution was autoclaved once more and then filtered through a 0.2-µm Stericup Quick Release System PES filter (Merck) into clean sterile Schott glass flasks and stored at room temperature in dark. The pH of SE was 5.28 ± 0.03. Its total organic carbon content (TOC) equaled 753 ± 49 mg C l⁻¹. A single batch of SE was used for all microcosms in this study. Analysis of soil parameters is described in the Supplementary methods.

Soil microcosm inoculation and culturing. Soil microcosms were inoculated with NatCom (four replicates) and SynCom (10 replicates) suspensions, and cultured either as a long-term single batch

incubation, or through multiple one-week growth and dilution cycles (Fig. 1A). Each microcosm initially comprised 90 g dry sterile soil matrix in a 500 ml screwcap glass bottle, amended with 10 ml community inoculum (at 10^7 cells ml^{-1} in SE, see above), thus resulting in ca. 10% gravimetric water content and 10^6 cells g^{-1} soil at start. The $\text{pH}(\text{H}_2\text{O})$ of the soil microcosms after inoculation was 8.62 ± 0.04 . Uninoculated soils (four replicates) amended with 10 ml sterile SE served as controls for potential contamination. To contrast community growth in liquid suspension, the same SynCom and NatCom inocula were grown directly in 10 ml SE in 50 ml sterile Falcon tubes (starting at 10^7 cells ml^{-1}), which were incubated at ambient temperature in the dark. After inoculation and before each sampling the soil microcosms were thoroughly mixed on a horizontal roller mixer (20 min at 80 rpm). SE-liquid microcosms were vortexed for 1 min every day.

In the long-term incubation series, samples (20 g) for community analysis (see below) were taken from each replicate microcosm after one week, two and six months. In the cycling regime, 11 g of the microcosm material were aseptically transferred after one week of growth to a fresh flask containing 80 g of dry sterile soil matrix. 9 ml of sterile SE was again added to maintain moisture content and replenish nutrient levels, thus resulting in ten-fold microcosm dilution upon each transfer. Flasks were again incubated for 1 week as before with intermittent roller-mixing. This incubation-dilution cycle was repeated eight times consecutively.

SE-liquid microcosms were sampled (2 ml) each week for community analysis, after which 1 ml was transferred to a fresh tube with 9 ml of sterile SE. Incubation and dilution were repeated for eight cycles, similar as for the soil microcosms with the cycling regime. A further SE-liquid control was prepared for the long incubation (one week, 2 and 6 months).

Chemical perturbation. In order to assess the effect of chemical perturbation on the resilience of the established communities, five of ten SynCom replicates (both soil+SE and SE-liquid) after the fifth transfer (see above) were exposed to toluene vapor during one week, as follows. After the inoculation with material from the previous cycle, heat-sealed 1 ml (for soils) or 0.2 ml (for SE-liquid) micropipette

tips were placed inside the microcosms, open at the top to the air, and filled with 100 μ l or 10 μ l pure toluene, respectively. These volumes are equivalent to a nominal concentration of 1.88 mM toluene, which will partition into the gas and aqueous phases in both systems. Microcosm flasks and tubes were tightly closed and incubated for 7 days with daily mixing (during each mixing, the toluene reservoir was briefly removed and then placed back). Samples were taken at day 7, and material from the exposed microcosms was again diluted as before into fresh soil+SE or SE-liquid, but without toluene. The non-exposed growth regime was repeated for another two cycles to study community recovery.

Community analysis. Samples of 20 g (soil+SE) or 2 ml (SE-liquid) were mixed with 20 ml of sterile pyrophosphate solution (see above) and vortexed for 1 min at maximum speed. The samples were left to stand for 1 min to settle soil particles, after which the supernatant was transferred aseptically to a new vial. An aliquot of 100 μ l of each sample supernatant (containing the cell suspension) was mixed with an equal volume of 4 M sodium-azide solution to fix the cells. Fixed samples were kept at 4°C until flow cytometry counting (see below).

The rest of the supernatant cell suspension (~19 ml) was centrifuged in a swing-out rotor (Eppendorf A-4-62 Swing Bucket Rotor) at 3200 $\times g$ for 10 min to pellet cells. The liquid was discarded and cell pellets were frozen at -80°C until DNA isolation. Cell pellets were thawed and DNA was purified using a DNeasy PowerSoil kit (Qiagen) according to manufacturer's protocol. The concentration of purified DNA was measured using a Qubit dsDNA BR Assay Kit (Invitrogen). DNA samples were stored at -20°C until library preparation (see below).

Flow cytometry. Cell suspensions were filtered using a 40- μ m nylon cell strainer (Falcon) and then fixed (see above). Fixed cell suspensions were serially diluted in sterile saline and stained with SYBR Green I for 15 min in the dark according to instructions of the supplier (Invitrogen). Stained cell suspensions were counted in 20 μ l sample volume at medium flow rate (60 μ l min⁻¹) using an ACEA

NovoCyt Green flow cytometer (OMNI Life Science Agilent). The SYBR Green I signal was measured in the FITC-channels of the instrument. Based on buffer controls, events with FSC-H-values above 50 and FITC-H above 350 were considered to potentially originate from microbial cells. Uninoculated microcosms, extracted and fixed in the same way, served to quantify cell-free (e.g., colloidal particles) background, which was subtracted from inoculated microcosm samples.

Identification of soil isolates. Each soil isolate was identified based on the near-full length 16S rRNA gene, amplified by PCR with Phusion U Hot Start PCR MasterMix (Thermo-Fischer Scientific) in presence of 0.5 mM betaine (Sigma-Aldrich) using universal bacterial primers (27F 5' AGAGTTTGATCCTGGCTCAG and 1492R 5' GGTTACCTGTTACGACTT, or 27F_deg 5' AGRGTTYGATYMTGGCTCAG and 1391R_v18 5' GACGGGCGGTGWGTRCA) (63). Amplified DNA was purified using Gel and PCR Clean-up kits (Macherey-Nagel) and single-end Sanger-sequenced with the corresponding forward primer at Eurofins Scientific. Sequences were compared to the SILVA database (version 132) using Blast (64) with default parameters for the genus level identification.

Community 16S rRNA gene amplicon sequencing. Aliquots of 10 ng purified DNA per sample were used to amplify the V3-V4 region of the bacterial 16S rRNA gene, following the Illumina 16S Metagenomic Sequencing Library protocol (https://support.illumina.com/documents/documentation/chemistry_documentation/16s/16s-metagenomic-library-prep-guide-15044223b.pdf), indexed with a set A Nextera XT Index Kit (v2, Illumina), quantified and pooled in equal amounts for sequencing. The pooled SynCom amplicon libraries were spiked with 25% PhiX control DNA and paired-end sequenced on an Illumina MiniSeq instrument with the mid-output flow cell (Illumina). NatCom libraries and a sample of the SynCom starting inoculum were sequenced on a MiSeq platform with 300 cycles MiSeq v3 paired-end sequencing at the Lausanne Genomic Technologies Facility. Given their known reduced composition, for SynCom samples only the V4-end reads were used for analysis. Raw sequence reads were quality

checked using FastQC 0.11.7 (65), cleaned and trimmed where necessary using Trimmomatic 0.36 (66). Primer sequences, ends with low quality and reads with poor quality score were removed. The quality was re-checked after trimming. A reference database of the inoculated SynCom members was created using the determined 16S rRNA gene sequences of each isolate (described above) and complemented by all unique sequence variants obtained from a MiSeq paired-end analysis of the SynCom inoculum. These reads were processed with QIIME 2 on a Unix platform (version qiime2-2018.8) (67), and grouped into taxonomic units at level 6 at 99% sequence identity by comparison to the SILVA database (version 132). Sequences were aligned using MUSCLE 3.8.1551 (68) and visualized using Jalview (69). Unique variable regions of 60 or 90 bp length were selected as identifier for each of the 21 SynCom strains. Strain abundances in the SynCom samples were then counted in the pools of quality-controlled sequence reads by searching for the unique selected sequence identifiers of each member in the reference database, using the bash command “grep”. The obtained counts were corrected for the number of 16S rRNA operons in the respective SynCom isolates genomes (to be described elsewhere). Relative abundances were then normalized to the total number of classified reads in each sample, which was further compared to differences in total cell count (as determined by flow cytometry) and the concentration of purified sample DNA.

Microbe Atlas comparison. All sample sequences were compared to a global background of soil communities from the Microbe Atlas Project database (MAPdb, <https://microbeatlas.org>). The raw 16S reads from all samples were standardized and quality-filtered using a custom C++ program employed internally by MAPdb and then mapped using MAPseq 1.2.6 (70) (reference database: MAPref v2.2; all other parameters kept at default) to obtain 97%-level OTU count tables compatible with MAPdb. Samples from MAPdb with meta-data annotations “soil” (main environment) or “rhizosphere” (sub-environment) were used for downstream analysis (110,928 samples total). Earth Microbiome Project (71) samples were identified based on accessions from <https://ebimetagenomics.github.io/blog/2019/04/17/Earth-Microbiome-Project/> and corresponding soil pH

values were extracted via the “sample_ph” field from accession-matched Sequence Read Archive (72) annotation files.

Simulation model. To test the effects of stochastic variations in starting numbers of rapidly growing members within complex communities, we deployed a recently developed community model that simulates substrate-limited Monod growth of large numbers of bacterial taxa simultaneously (73). The model was seeded with 200,000 individual cells sampled with a weighted probability distribution from the measured relative abundances of 314 major taxa in a soil sample. Growth rates were attributed between 0.01 and 0.4 h⁻¹ according to the log₁₀ relative taxa abundance at start, except for five taxa with subsampled starting numbers between 0 and 10 (of 200,000 cells in total) that were given growth rates of 0.55, 0.25, 0.8, 0.6 and 0.35 h⁻¹. Growth was allowed to proceed until all carbon was depleted, after which the final community was subsampled to 200,000 cells (to resemble a sequenced sample with 2×10⁵ reads). Relative and stacked taxa abundances were plotted within these subsampled data sets. Simulations were repeated five times independently.

Statistical analyses. Data were analysed using R 3.6.1 (R Core Team, 2019) and the R packages *vegan* (74), *ggplot2* (75), *phyloseq* (76), *reshape* (77) and also using GraphPad Prism (version 9.0.0 for Mac OS X). The trends of microcosm total cell densities (as measured by flow cytometry) were compared using ANCOVA ($n = 4$ -10 replicates per condition). Absolute abundances per SynCom community member were calculated from their relative (sequence) abundance times the measured total community size per replicate (from flow cytometry). The influence of culturing environment (e.g., soil, liquid) on community yield was compared using one-tailed t-tests. Differences in DNA yields were compared using a one-way ANOVA with post-hoc Tukey’s multiple comparisons test. The inter-replicate variability was expressed by the Bray-Curtis replicate distance from the community centroid. Effects of conditions were compared using one-way ANOVA with post-hoc Tukey’s multiple comparisons test. Alpha diversity was computed as community richness and Shannon indices.

Communities at different time points and treatments were compared by non-metric multidimensional scaling (NMDS) using Bray-Curtis distance values of normalized relative community member abundances. Multivariate dispersion of the data was examined using the *betadis* function from *vegan*. Adonis (MANOVA with 999 permutations) was used to assess the differences between groups based on the output of *vegdist* (Bray-Curtis distances). The effect of toluene exposure on community cell densities was assessed using a Wilcoxon matched-pairs signed rank test. The effect of time and toluene exposure on community richness and Shannon values was assessed using two-way ANOVA. Clustered heatmap and UMAP (78) projections were generated from Bray-Curtis distance matrices using Julia 1.6.0 (79) and the *Distances.jl* package ((80), version 0.10.3). UMAP projections were computed using the *UMAP.jl* package (<https://github.com/dillondaudert/UMAP.jl>, version 0.1.8; parameters: *n_neighbors*=500, *min_dist*=1.5, *spread*=15, *epochs*=2000). Scatter plots were produced using Python 3.9.1 (81) and the *seaborn* package ((82), version 0.11.0).

Database access. The NatCom and SynCom sequencing data are available from the Short Read Archives under BioProject number PRJNA767350.

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Author contributions

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Table 1. Taxonomy of selected strains for the synthetic soil community (SynCom).

No.	Genus	Class	Phyla
1	Microbacterium	Actinobacteria	Actinobacteria
2	Mucilaginibacter	Bacteroidia	Bacteroidetes
3	Curtobacterium	Actinobacteria	Actinobacteria
4	Variovorax	Gammaproteobacteria	Proteobacteria
5	Flavobacterium	Bacteroidia	Bacteroidetes
6	Cellulomonas	Actinobacteria	Actinobacteria
7	Tardiphaga	Alphaproteobacteria	Proteobacteria
8	Devosia	Alphaproteobacteria	Proteobacteria
9	Mesorhizobium	Alphaproteobacteria	Proteobacteria
10	Burkholderia	Betaproteobacteria	Proteobacteria
11	Pseudomonas	Gammaproteobacteria	Proteobacteria
12	Luteibacter	Gammaproteobacteria	Proteobacteria
13	Chitinophaga	Bacteroidia	Bacteroidetes
14	Lysobacter	Gammaproteobacteria	Proteobacteria
15	Pseudomonas	Gammaproteobacteria	Proteobacteria
16	Rhodococcus	Actinobacteria	Actinobacteria
17	Caulobacter	Alphaproteobacteria	Proteobacteria
18	Cohnella	Bacilli	Firmicutes
19	Rahnella	Gammaproteobacteria	Proteobacteria
20	Phenylobacterium	Alphaproteobacteria	Proteobacteria
21	Bradyrhizobium	Alphaproteobacteria	Proteobacteria

Figure 1. Characteristics of the soil culturing system. (A) Freshly washed soil communities (NatCom) or synthetic composed soil community (SynCom, 21 species) were used to inoculate 4 and 10 replicate sterile soil microcosms (each 100 g soil, 10^6 cells g^{-1} at start), respectively. Microcosms were incubated for 7 days and then diluted into sets of fresh sterile microcosms (1:10, w/w). This growth cycling was repeated for a total of 8 cycles. The first microcosms were continued and sampled after 2 and 6 months. (B) Measured pH-H₂O for soil before (black), after (dark grey) autoclaving and supplemented with SE (light grey), as well as the SE alone (grey). (C) Values of cation exchange capacity in $cmol_c kg^{-1}$ of soil for soil before, after autoclaving and supplemented with SE. Bars show the mean from 3 replicates $\pm$ one SD. (D) Organic matter levels evaluated by UV/Vis measurements as total fluorescence (F_{tot}) (filled bars, left axis, the same color scheme as in (C)) or Napierian absorbance (no fill, right axis, the same color scheme) for the materials used in the soil microcosms. Bars show means from 12 replicates $\pm$ one SD. (E) Inferred composition of organic matter by PARAFAC, according to the six defined groups of compounds in Ref. (39): C1, UVA Humic-like; C2, UVA-Humic like; C3- UVC-

1 Humic-like; C4, Tyrosine-like; C5, UVA Humic-like and C6, Tryptophane-like. Bars are means from $n =$
2 12 replicate measurements.

Figure 2. Development of synthetic and natural soil microbial communities in soil microcosms. (A)

Figure 3. Community succession and composition of NatComs. (A) Mean $\log_{10}$ -transformed total read-normalized (5×10^4) taxa abundances in the soil inoculum, after all the 8 one-week growth cycles, and in the longer-term incubation timepoints (2 and 6 months). Abundance bars positioned according

to taxa numbering from the OTU list (SILVA, above 99% similarity), with background color representing phyla affiliation (Roman numbering, according to legend). Numbers within panels show mean taxa richness $\pm$ one *SD* ($n = 4$ replicates). Magenta bars in the CYCLE-1 data point to taxa not detected in the inoculum. **(B)** Non-metric multidimensional scaling ordination of NatCom succession in soil + SE (magenta area), or in liquid SE suspension (cyan area; T1–T8, weekly transfers; L2, L6, two and six months incubations). Ordination plot based on Bray-Curtis distances. **(C)** Compositional variation (shown as $\log_{10}$ -normalized abundance heatmap) among the four NatCom replicates (REP A–D) after the first growth cycle. Numbers above refer to taxa within order-levels as specified on the right. **(D)** Mean standard error of replicate variation (REP A–D after one week) at OTU-level (mean of means grouped within corresponding order, pink) or at order-level (blue; purple is where both OTU- and order-values overlap). Note how order-level variation is lower than OTU-variation. Numbers refer to order in (C). -, single OTU in order; not specified. **(E)** NatCom pairwise sample comparison, clustered by average-linked Bray-Curtis distances (color scale). Inoculum (soil 1-3) and replicates (R1–4) are highlighted by different colors on the top, growth cycles (T1–T8) or long-term incubations (L2, 2 mo; L6, 6 mo) in small fonts on the right. Note the strongly maintained replicate signatures (e.g., replicate 2). **(F)** Mean (bars) and individual replicate (grey to black dots, $n = 4$) grouped phyla composition of NatCom inoculum (orange), after the first growth cycle (CYCLE 1, 1 wk), the 8th (CYCLE 8), and after 2 and 6 months (mo).

Figure 4. Succession and stabilization of a synthetic soil community over multiple growth cycles and long-term incubation. (A) Class attribution of the 172 isolated soil bacterial strains, and of the selected 21 strains of the SynCom. **(B)** Changes in mean SynCom richness (magenta line, $\pm$ one SD in light color background, $n = 10$ replicates) and **(C)** in mean Shannon indices (box plots, $n = 10$; except T6-T8; $n = 5$ replicates) throughout the 8 growth cycles in soil + SE (T1–T8), and during long-term incubation (L2, L6; 2 and 6 months, mo). **(D)** Stacked mean relative abundances (in percentage, $n = 10$ replicates) of SynCom members (legend on the right) from inoculation to the last growth cycle, and upon long-term incubation. **(E)** Non-metric multidimensional scaling of normalized SynCom compositions according to their Bray-Curtis distances. Black dots show the community centroids; colored dots are individual replicates.

Figure 5. NatCom and SynCom community signatures. (A) Environmental signature of NatComs and SynComs. Map shows a UMAP projection of SynCom and NatCom samples together with 110,928 soil communities (dots) extracted from the Microbe Atlas Project (70), based on Bray-Curtis distances and color coded along their environmental origin, or **(B)** overlaid with soil pH, extracted from the Earth Microbiome Project (71). **(C)** SynCom and NatCom relative abundances at phyla and class levels (Proteobacteria only). **(D)** Interreplicate variability of SynCom and NatCom replicates, shown here as individual Bray-Curtis distances to the corresponding community centroid. Boxplots show 25th, median and 75th percentiles, with whiskers indicating 1.5× the interquartile range.

Figure 6. Community resilience upon chemical perturbation. (A) Mean (lines) and replicate (dots) SynCom size changes (in cells ml⁻¹ soil liquid phase) in toluene-exposed (grey) versus non-exposed (orange) microcosms (each 5 replicates). Toluene exposure during 1 week of the fifth growth cycle. P-value refers to comparison of cell densities after the 6th-8th cycles between exposed and non-exposed communities in a Wilcoxon matched-pairs signed rank test. **(B)** Mean (bars) and replicate (dots) richness in toluene-exposed versus non-exposed SynComs. P-values from two-way ANOVA for toluene exposure. **(C)** as B but for Shannon index. **(D)** Changes in absolute abundances (calculated from individual relative sequence abundances and total community size by flow cytometry) of selected

1 SynCom members with and without toluene exposure (two-sided t-test, grouped T6–T8 values). **(E)**
2 Interreplicate variability, expressed as average distance of replicates to community centroid. P-value
3 from ANOVA of exposed versus non-exposed centroid distances. **(F)** Toluene-exposure effect on
4 SynCom compositions after the 6th–8th growth cycles (NMDS based on Bray-Curtis community
5 distances). Sample abbreviations (e.g., T1) as before; black dots, community means; grey dots,
6 toluene-exposed SynCom replicates during T5; orange to brown dots; non-exposed SynCom.
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