New Phytologist

Research

Simplification of soil biota communities impairs nutrient recycling and enhances above- and belowground nitrogen losses

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Received: *11 May 2023*
Accepted: *18 August 2023*

New Phytologist (2023)
doi: 10.1111/nph.19252

Key words: denitrification, ecosystem functioning, maize, metagenome, nitrous oxide, nutrient leaching, soil biodiversity.

Summary

- Agriculture is a major source of nutrient pollution, posing a threat to the earth system functioning. Factors determining the nutrient use efficiency of plant–soil systems need to be identified to develop strategies to reduce nutrient losses while ensuring crop productivity. The potential of soil biota to tighten nutrient cycles by improving plant nutrition and reducing soil nutrient losses is still poorly understood.
- We manipulated soil biota communities in outdoor lysimeters, planted maize, continuously collected leachates, and measured N_2O- and N_2-gas emissions after a fertilization pulse to test whether differences in soil biota communities affected nutrient recycling and N losses.
- Lysimeters with strongly simplified soil biota communities showed reduced crop N (−20%) and P (−58%) uptake, strongly increased N leaching losses (+65%), and gaseous emissions (+97%) of N_2O and N_2. Soil metagenomic analyses revealed differences in the abundance of genes responsible for nutrient uptake, nitrate reduction, and denitrification that helped explain the observed nutrient losses.
- Soil biota are major drivers of nutrient cycling and reductions in the diversity or abundance of certain groups (e.g. through land-use intensification) can disrupt nutrient cycling, reduce agricultural productivity and nutrient use efficiency, and exacerbate environmental pollution and global warming.

Introduction

Nitrogen (N) is a fundamental nutrient for all living organisms and can limit plant productivity and impact food security around the globe (Elser *et al.*, 2007; Gruber & Galloway, 2008). At the same time, excess N inputs into the biosphere through anthropogenic activities lead to environmental problems including the eutrophication of drinking water and the emission of greenhouse gases (Schlesinger, 2009; Steffen *et al.*, 2015). In addition, the current use of industrially produced reactive N in cropping systems is highly inefficient. It has been estimated that *c.* 50% of N fertilizers applied globally are not taken up by crops and are at risk of being lost to the environment, representing a loss of valuable resources and causing a multitude of adverse effects on ecosystems (Galloway *et al.*, 2003; Liu *et al.*, 2010).

Soil biota act as catalysts for major parts of the N cycle including the release of mineral N from organic materials and various N assimilation or transformation processes, such as N-fixation, nitrification of ammonium into nitrate, or denitrification of nitrate into N_2O and N_2. These processes result in the transformation of N into different chemical forms that determine whether N can be taken up by plants, remains in soil, or is at risk of being lost to the environment through leaching or in gaseous form (Niu *et al.*, 2016). In addition, soil biota themselves act as an important temporal storage pool for N. Substantial knowledge exists on the biogeochemical basis of N cycling, the enzymes involved, and the conditions for the expression of the respective genes, and many of the biota involved (Kuypers *et al.*, 2018). A high number of new pathways and ecological processes involved in N cycling have been discovered in recent years (Sanford *et al.*, 2012; Jones *et al.*, 2014; Daims *et al.*, 2015; Selbie *et al.*, 2015; Van Kessel *et al.*, 2015; Kits *et al.*, 2017; Kitzinger *et al.*, 2019; Hestrin *et al.*, 2021; Mooshammer *et al.*, 2021; Li *et al.*, 2023), suggesting that the N cycle is more complex than believed in the past.

Many agricultural management practices have been shown to exert adverse effects on the diversity, abundance, and complexity

of entire soil biota communities (de Vries *et al.*, 2013; Tuck *et al.*, 2014; Tsiafouli *et al.*, 2015; Banerjee *et al.*, 2019; Riedo *et al.*, 2021; Edlinger *et al.*, 2022). The relevance of soil biota communities for N cycling implies that their simplification, that is reductions in their abundance, diversity, or complexity (e.g. through land-use intensification), could compromise N cycling on the ecosystem scale. This has been indicated by a number of correlative field studies, which suggested a high importance of soil biota communities for nitrogen cycling processes (de Vries *et al.*, 2013, 2015; Zheng *et al.*, 2019) and overall soil multifunctionality (Delgado-Baquerizo *et al.*, 2020). A microcosm experiment showed that reductions in soil microbial diversity affect soil N cycling indicating the importance of soil microorganisms for N transformations (Philippot *et al.*, 2013).

A particularly influential group of soil organisms that have often been shown to affect nutrient cycling processes are the plant symbiotic arbuscular mycorrhizal fungi. Besides their ability to improve plant nutrition (Smith & Smith, 2011), they have been shown to affect nitrogen and phosphorus cycling with effects on nutrient losses from soil (Cavagnaro *et al.*, 2015; Qiu *et al.*, 2021).

In an earlier study, we demonstrated that simplification of soil biota communities resulted in enhanced nitrogen leaching (Bender & van der Heijden, 2015). However, whether the simplification of soil biota communities also influences aboveground nitrogen losses through gas emitting processes, including N_2O and N_2 production, and the overall N budget of agroecosystems is still unclear. So far, very few studies have comprehensively assessed above-and belowground nutrient losses simultaneously. Here, we present direct evidence that the simplification of soil biota communities can strongly affect nutrient cycling and nutrient use efficiency in plant–soil systems. We provide a comprehensive assessment of crop nutrient uptake and N losses from soil through leaching and gaseous emissions using an outdoor lysimeter setup simulating an agricultural cropping system. Lysimeters comprised a volume of 230 l and were filled with sterilized agricultural soil and inoculated with two soil community treatments representing either a moderately simplified soil community (MSC, soil filtration to < 400 µm) or strongly simplified soil community (SSC, soil filtration to < 12 µm). Previous studies have shown that such filtering approaches can result in the simplification of soil biota communities (Wagg *et al.*, 2014, 2019). Soil biota communities in the SSC treatment showed lower microbial diversity and abundance of AMF than communities in the MSC treatment. Since both treatments were subjected to a filtering treatment removing larger soil biota (e.g. earthworms and larger soil invertebrates), they both represented a simplification compared with natural soil biota communities. Maize was planted in the lysimeters and grown for one growing season. We assessed nutrient losses through leaching during the entire course of the study and monitored N_2O and N_2 fluxes after a fertilization pulse using the nitrogen isotope ^{15}N. Corn yield, as well as crop and soil nutrient contents were assessed. Using metabarcoding approaches and metagenomics sequencing, we related the differential distribution of N in the system to soil biota communities and their functional properties. We calculate an N budget, assess inputs and outputs (losses), calculate different efficiency indicators, and relate N inputs to N uptake into crop biomass, or losses to the environment. We show that the simplification of soil biota communities can lead to a disruption of nutrient cycling with severe consequences for N use efficiency in cropping systems. We prove that soil biota play a crucial role for agricultural nutrient cycling, N use efficiency, greenhouse gas emissions, and potential pollution of waterways.

Materials and Methods

Soil biological inoculum

We produced two different inocula of soil biota communities in the greenhouse as described in detail in the Supporting Information Methods S1 (see also Fig. S2; Table S1). The moderately simplified community inoculum (MSC) was produced to potentially contain all soil biota < 400 µm and the strongly simplified community inoculum (SSC) potentially contained all soil biota < 12 µm extracted from the same field soil used to fill the lysimeters (to be described later). Biological analyses of inocula were performed as described in Methods S1. Inocula differed significantly in prokaryotic and eukaryotic diversity, the abundance of arbuscular mycorrhizal fungi, and some microbiological indicators (Fig. S2; Table S2).

Lysimeter setup

The experimental setup largely represents settings previously reported in Bender & van der Heijden (2015). We made use of 16 outdoor lysimeters, each comprising a volume of *c.* 230 l (*d*: 60 cm, *h*: 80 cm) and containing a drainage hole in the bottom for collection of water percolating through the soil profile (see Fig. S3a). We collected 6 m^3 of an organically managed pasture soil from a site in Zürich, Switzerland (47.431231°N, 8.527289°E), characterized as *Calcaric cambisol*. Soil from 0 to 30 cm and from 30 to 80 cm depth was collected separately and defined as topsoil and subsoil, respectively. Soil was processed and sterilized as described in Methods S1.

To fill the Lysimeters, first, 90 l of subsoil was added, followed by 90 l of topsoil. Topsoil was thoroughly mixed with 10.8 l of the respective soil biological inoculum and a microbial wash and covered with 2 l of sterile soil. A barrier of transparent Plexiglas of 30 cm height was attached around each lysimeter to reduce the risk of contamination (Fig. S3b). After filling the lysimeters, excess nutrients resulting from soil sterilization were flushed out through excessive watering. See Methods S1 for detail.

The 16 lysimeters were arranged in two blocks of eight lysimeters, each. On 23 May 2014, nine maize seeds (*Zea mays* L., var. Laurinio, RAGT, FR) were planted in each lysimeter, and soil samples were taken. After 2 wk, maize plants were thinned to three per lysimeter. Soil properties at the start of the experiment are shown in Table S3. A chronological overview of the different phases and sampling procedures of the study is presented in Fig. S4.

Leachate collection and analyses

A hole in the bottom of the lysimeters with a closable valve connected to a PVC tube leading to a preweighed container (Fig. S3a) allowed leachate collection. Leachate collection occurred continuously over the course of the study, each time natural leaching events occurred, that is when soil water started accumulating in the containers below the lysimeters, for example after sufficient rain. Concentrations of NO_3, NH_4, NO_2–N, total dissolved N (TDN), PO_4–P, and SO_4 in leachates were assessed. The difference between TDN and mineral N (NO_3–N, NO_2–N, and NH_4–N) was considered dissolved organic N (DON). All nutrient concentrations were multiplied by the leachate volume at each sampling event, to calculate the total amount of nutrients lost per lysimeter. See Methods S1 for details.

Fertilization

Through soil sterilization, nutrient availability in lysimeters was expected to be rather high and fertilization only followed the purpose of generating detectable N_2 and N_2O gas fluxes following an approach used earlier (Stevens & Laughlin, 1998). Shortly before fertilizer application, the water content in all lysimeters was raised to maximum water-holding capacity. A fertilizer solution containing 60 atom % ^{15}N-enriched KNO_3 was applied to the lysimeter surfaces. The morning after fertilization, valves at the drainage holes were closed and each lysimeter was watered with an additional 10 l, simulating a heavy summer rain event corresponding to 36 mm of precipitation. These simulated conditions are comparable to a poorly drained Swiss soil during a wet summer period, providing conditions conducive for denitrification. See Methods S1 for further details.

Gas measurements

Gas measurements started directly after fertilization using small plastic chambers inserted in the soil. Two different methods were applied simultaneously: (1) a continuous-flow TEI46c-automated N_2O analyzer (Thermo Fisher Scientific, Waltham, MA, USA) allowed assessing N_2O emissions and (2) gas chromatography combined with isotope ratio mass spectrometry (GC-IRMS) allowed assessing N_2O and N_2 fluxes and their ^{15}N signal. Gas measurements started 3 h after watering and 24 h after fertilization on 14 July and were performed daily, until 19 July. The last sampling was conducted 2 d later, on 21 July.

GC-IRMS analyses were performed at the Agri-Food and Biosciences Institute, Belfast, UK, using a Europa Scientific 20–20 Stable Isotope Analyzer interfaced to a Europa Scientific Trace Gas Preparation System with Gilson autosampler as described in Stevens & Laughlin (1998). See Methods S1 and Fig. S5 for detail and additional discussion.

Soil sampling

Analyses of general soil characteristics and microbial biomass N were performed on five composite soil samples per lysimeter (0–15 cm depth) at the time points of sowing and harvest. At harvest, microbial biomass N was determined from freshly sampled soil stored at 4°C overnight, while at sowing, microbial biomass N was determined from soil that had been frozen for several weeks. Due to these different storage conditions, both data may not be directly comparable. At the time point of harvest, in addition, intact soil cores of 5 cm diameter were taken from 0 to 5 and 5 to 10 cm depth for soil fauna extraction using Berlese funnels. After extraction, collembola and mites were identified and quantified at the species level, using microscopy. Approximately 70 g of moist soil was used to extract nematodes using a Baermann funnel method (Baermann, 1917). Soil samples for DNA extraction were collected at several time points during the experiment of which five time points (at sowing, fertilization, 120 h after fertilization, 144 h after fertilization, and at harvest) were chosen to be analyzed. See Methods S1 for details.

Plant harvest and analyses

Maize plants were harvested on 6 September by cutting them at 10 cm above soil surface and total fresh and dry weight was determined. A 30 × 30 × 30 cm cube of soil around each maize stem was extracted, and roots were collected from the soil and washed. Root fresh and dry weight was determined, and subsamples for the assessment of AMF root colonization were taken. Macronutrient concentrations (N, P, and K) were determined in shoots and roots. Plant biomass on a kg ha^{-1} basis was determined by extrapolating biomass per lysimeter surface area. Root samples were assessed for arbuscular mycorrhizal fungal root colonization after staining using a modified grid-line intersection method on 100 intersections per sample (McGonigle *et al.*, 1990). See Methods S1 for details.

Soil DNA analyses

Our focus was to achieve high sequencing depth for the metagenomic analysis (to be described later). Therefore, we randomly selected four replicate lysimeters per treatment and extracted DNA from samples collected at five different time points during the study, resulting in a total number of 40 samples. DNA samples were used for metabarcoding of bacterial and fungal communities, for metagenomics analysis of the functional properties of soil biota and the quantification of 16S rRNA and ITS genes in soil.

Metabarcoding of bacterial and fungal communities

Amplicon next-generation sequencing of the 16S rRNA gene of bacteria and the ITS2 region of eukaryotes was performed on an Illumina MiSeq platform (Illumina Inc., San Diego, CA, USA) to assess prokaryotic and eukaryotic diversity in the samples. The 16S rRNA amplicon library was prepared using the universal eubacterial primers 27f and 357r covering the V1–V2 hypervariable regions of the 16S rRNA gene (Klindworth *et al.*, 2013).

For the ITS amplicon library, the PCR primers ITS3NGS and ITS4Uni covering the ITS2 region (Tedersoo & Lindahl, 2016),

covering fungi and some other eukaryotic groups, were used. See Methods S1 for details on library preparation.

Processing of the 16S rRNA and ITS raw sequences was conducted with QIIME2 (Bolyen *et al.*, 2019) and using the DADA2 plugin (Callahan *et al.*, 2016). The 16S rRNA gene ASV reference sequences were taxonomically assigned using the SILVA database (v.138; Quast *et al.*, 2012), and ITS ASV reference sequences were taxonomically assigned using the UNITE full eukaryotic database (v.9; Abarenkov *et al.*, 2010). See Methods S1 for details.

After all filtering steps, the prokaryotic (16S rRNA gene) community profiling yielded a total of 1414 843 high-quality sequences (ranging from 17 685 to 64 938 sequences per sample, median: 35 185).

For ITS sequences, the community profiling yielded a total of 108 426 sequences. A big fraction of sequences was found to be plant DNA. After filtering, a total of 74 155 sequences (ranging from 8 to 32 459 sequences per sample, median: 452.5) could be assigned to eukaryotic organisms (fungi, protists, and nematodes). ITS data were rarefied to 200 sequences per sample for all further analyses, whereby four samples were removed due to low sequence counts. Rarefaction curves for 16S and ITS sequences are shown in Fig. S6.

Shotgun metagenomics

Metagenome sequencing was performed on samples collected at fertilization and 120 h after fertilization. Metagenomic libraries were constructed using the NEBNext® Ultra II DNA Library Prep Kit for Illumina (Illumina Inc.). Libraries were diluted to 4 nM, pooled equimolar and shotgun sequenced using the Illumina MiSeq platform (Illumina Inc.) using paired-end mode (2 × 300 bp).

Raw sequences were processed as described in the Methods S1. Sequences were submitted to the SQUEEZEMETA v.1.5.0 (Tamames & Puente-Sánchez, 2019) pipeline for functional annotation in sequential mode. DIAMOND (Buchfink *et al.*, 2015) performed searches against the taxonomic and functional databases GenBank nr NCBI database, eggNOG (Huerta-Cepas *et al.*, 2016), and KEGG (Kanehisa & Goto, 2000). HMMER3 (Eddy, 2009) was also used for classification against the PFAM database (Finn *et al.*, 2014). Taxonomic assignments of genes were carried out using the LCA algorithm and the DIAMOND results against the GenBank nr database. Of a total of 23 245 763 sequences, 147 689 could be annotated to 42 genes involved in N cycling and 91 541 sequences could be annotated to 34 genes involved in P transformation. Genes detected in less than one-third of samples were removed from further analysis. Nonpareil curves representing the coverage and sequence diversity of the metagenomes are shown in Fig. S7. See Methods S1 for details.

Sequencing raw data from this study are available at the sequence read archive (http://www.ncbi.nlm.nih.gov/sra) at the National Center for Biotechnology Information under accession no. PRJNA648050. See Supporting Information for details.

Quantification of bacterial and fungal abundance

Quantitative PCR was performed to quantify bacterial 16S rRNA gene copy numbers in soil using the primers 338F and 518R (Muyzer *et al.*, 1993; Suzuki & Giovannoni, 1996) and fungal ITS gene copy numbers using the primers ITS1F and ITS2R (White *et al.*, 1990).

Statistics

Statistical analyses were conducted using the software R, v.4.2.1 (R Core Team, 2022). Effects of inoculation treatments on plant yield, nutrient concentrations, and cumulative leaching and gaseous nutrient losses were analyzed using mixed-effects models in the R package NLME (Pinheiro *et al.*, 2023) including treatment as fixed, and Block as random effect. For analyses of diversity indices, sampling time point was included as fixed effect and the experimental unit (lysimeter ID) within Block added as random effect. *Post hoc* analyses were conducted to test for differences between treatments at single time points using the 'emmeans' function from package EMMEANS (Lenth, 2022). Model residuals were checked for normality and homoscedasticity by plotting fitted values against residuals.

P-values were FDR adjusted for multiple comparisons using the Benjamini–Hochberg procedure (Benjamini & Hochberg, 1995). Principal coordinate analysis (Pcoa) of microbial communities was conducted, and diversity indices were calculated using the R package PHYLOSEQ (McMurdie & Holmes, 2013). PERMANOVA was conducted using the 'adonis2' function with marginal sums of squares in package VEGAN (Oksanen *et al.*, 2022). Since the adonis2 function using marginal sums of squares does not report test results for main effects when an interaction is included in the model, we additionally ran the model for main effects only, without interaction. Differential abundance analysis of microbial communities in MSC and SSC treatments was conducted using the R package DESEQ2 (Love *et al.*, 2014). Data used for this publication are available in Dataset S1.

Results

Effects on maize performance

Treatments significantly affected maize growth and nutrient uptake (Fig. 1; Table S4). Maize yield was 13% lower in the strongly simplified community treatment (SSC, 39.1 t ha^{-1}) compared with the moderately simplified community treatment (MSC, 45.1 t ha^{-1}). Similarly, concentrations of plant N, P, and K were significantly reduced by 20%, 58%, and 24%, respectively, in the SSC treatment.

Plant N uptake amounted to 608 kg N ha^{-1} in the MSC treatment compared with 489 kg N ha^{-1} in the SSC treatment, while plant P uptake amounted to 114 kg P ha^{-1} compared with 49 kg P ha^{-1}, considering all plant parts, including roots. The N : P ratio at harvest differed accordingly, showing a value of 5.4 in the MSC treatment and of 10.1 in the SSC treatment,

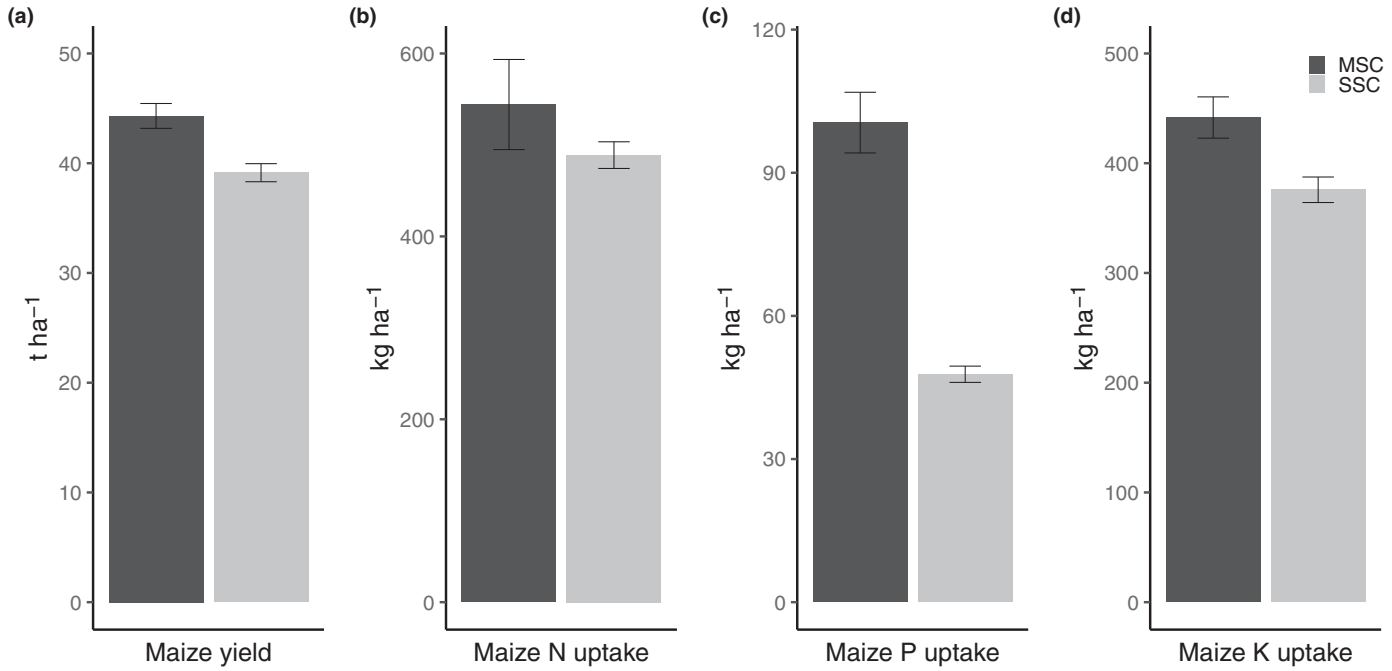

Fig. 1 Maize (*Zea mays*, L.) yield (a) and total amounts of plant nutrients (b–d) taken up over the entire growing season from lysimeters inoculated either with a moderately simplified (MSC) or strongly simplified (SSC) soil community inoculum. Error bars = ±1SE (n = 8).

indicating that N was limiting plant growth in both treatments, but N limitation was stronger in the MSC treatments (Koerselman & Meuleman, 1996).

Nutrient leaching losses

Over the entire growing season, a total of 248 kg N ha^{-1} was lost through leaching in the MSC treatment, while in the SSC treatment, total N leaching losses amounted to 409 kg N ha^{-1}, representing an increase of 65% (Table S4). Around 25% of N leaching occurred in organic form in both treatments (Fig. 2). PO_4–P leaching amounted to 0.12 kg P ha^{-1} in the MSC treatment and to 0.04 kg PO_4–P ha^{-1} in the SSC treatment, corresponding to a reduction of 67% (Table S4).

Gaseous N emissions

Lysimeters received a ^{15}N-enriched fertilization pulse in July and gaseous N emissions were monitored over a period of 8 d. N_2O fluxes started increasing between 24 and 48 h after fertilization. In the MSC treatment, fluxes peaked at 72 h after fertilization at a rate of 9.83 g ha^{-1} d^{-1} and subsequently declined until reaching a flux of 1.89 g ha^{-1} d^{-1} after 8 d. In the SSC treatment, N_2O fluxes peaked after 120 h at a rate of 23.75 g ha^{-1} d^{-1} and subsequently declined, reaching a flux of 3.29 g ha^{-1} d^{-1} after 8 d (Fig. 3a). Over the course of the 8-d period, a total of 36.6 g ha^{-1} N_2O–N was emitted from the MSC treatment, while a total of 81.9 g ha^{-1} N_2O–N was emitted from the SSC treatment, representing an increase of 124% (Table S4).

In the MSC treatment, N_2 fluxes started to increase between 48 and 72 h after fertilization, peaked at 120 h after fertilization

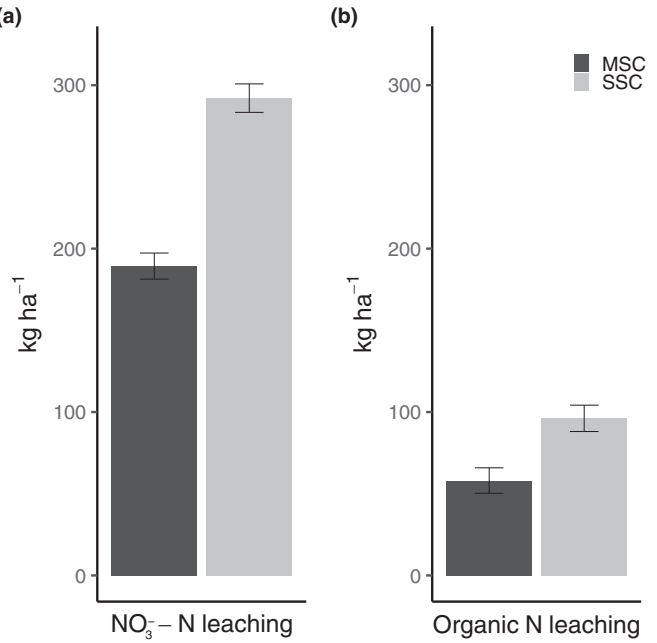

Fig. 2 Cumulative (a) Nitrate and (b) organic nitrogen leaching losses over the entire growing season from lysimeters either inoculated with a moderately simplified (MSC) or strongly simplified (SSC) soil community inoculum. Error bars = ±1SE (n = 8).

at a rate of 1743 g ha^{-1} d^{-1}, and subsequently declined to reach values below the detection limit at 8 d after fertilization. In the SSC treatment, N_2 fluxes also started to increase between 48 and 72 h after fertilization, peaked at 120 h after fertilization at a level of 2866 g ha^{-1} d^{-1}, and subsequently declined until reaching a flux of 119 g ha^{-1} d^{-1} after 8 d (Fig. 3b). Cumulative N_2

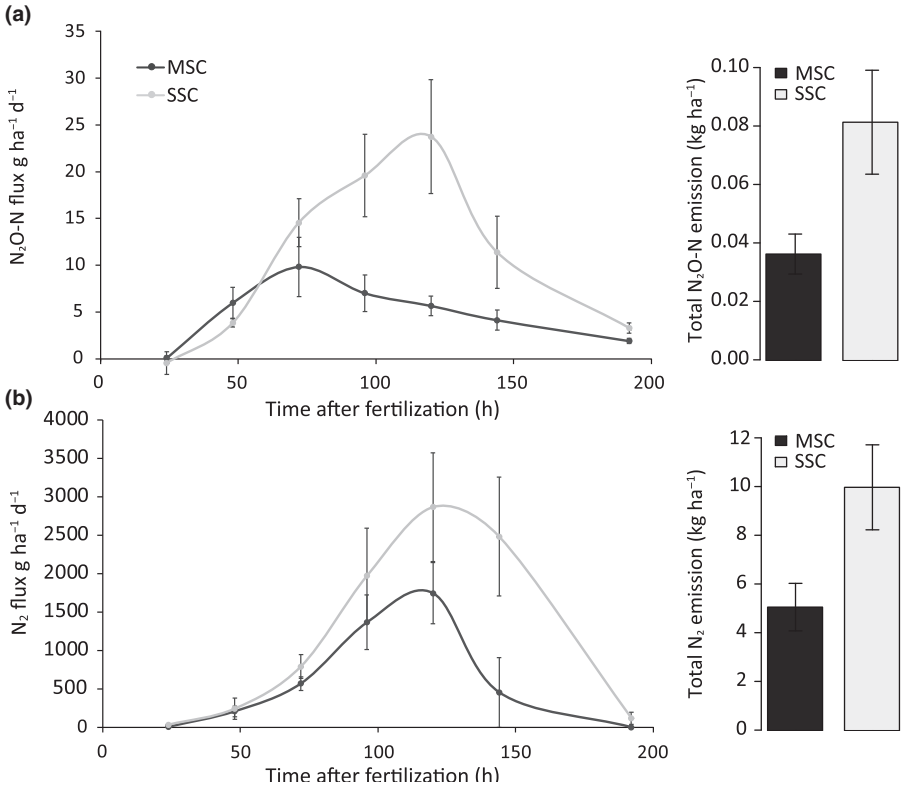

Fig. 3 N_2O–N fluxes (a) and N_2 fluxes (b) from lysimeters inoculated either with a moderately simplified (MSC) or strongly simplified (SSC) soil community inoculum measured over a period of 192 h after a fertilization pulse. The bar graphs represent cumulative gaseous N losses obtained though integration of flux curves. Error bars = ±1SE ($n = 8$).

emissions amounted to 5.09 kg ha^{-1} in the MSC treatment and 10.03 kg ha^{-1} in the SSC treatment, indicating an increase of 97% (Table S4).

Nitrogen use efficiency calculations

We used data on soil reactive N pools (soil mineral N and microbial biomass N), fertilizer addition, plant N contents, and N losses though leaching and gaseous emissions to calculate an overview of the different N pools for each experimental unit (Table 1a). Average N inputs (N pools at sowing + fertilizer addition) did not differ significantly between both treatments (Table S5). Mineral N contents found in soil at harvest amounted to 51.7 kg N ha^{-1} in the MSC treatment and were significantly higher in the SSC treatment, reaching levels up to 73.1 kg ha^{-1}. Microbial biomass N at harvest came to 146 kg ha^{-1} in the MSC treatment and 134 kg ha^{-1} in the SSC treatment but this difference was statistically not significant (Table S5). A total of 253 kg N ha^{-1} was lost through leaching and gaseous emissions from the MSC treatment, compared with 419 kg N ha^{-1} lost in the SSC treatment. Of the total amount of N detected in lysimeters, 58% and 44% were taken up by plants in the MSC and SSC treatments, respectively, while 24% and 38% were lost to the environment (Table 1b).

We defined *N use efficiency* (NUE) as the amount of plant N uptake per unit N input (mineral soil N at sowing plus mineral N from fertilizer) and *N use inefficiency* (NUI) as the amount of N lost through leaching or as gas per unit N input. While NUE was only slightly and not significantly reduced (−8%) in the SSC treatment, NUI was significantly higher (+86%) in the SSC compared with the MSC treatment. We calculated the *net N use efficiency* (NNE) in our model system by subtracting NUI from NUE. This value gives an indication for the net plant N uptake without corresponding N losses to the environment. NNE was 77% lower in the SSC compared with the MSC treatment (Tables 2, S5).

Soil biological parameters

At harvest, average AM fungal root colonization differed significantly between treatments and amounted to 70.5% (±3.4%; $F_{1,13} = 482.03$, $P < 0.001$) of root length colonized in the MSC treatment. In the SSC treatment, five replicates showed no sign of AM fungal colonization, while three replicates showed levels between 5% and 7%, resulting in an overall average root colonization of 2.5% (±1.1%). The abundance of soil arthropods and nematodes assessed at harvest was low and showed no significant differences between treatments (Table S6). This result might be related to the fact that at the time point of sampling in August, air temperature was quite high. It seems likely that soil animals had moved to deeper soil layers (> 10 cm) and escaped sampling.

We performed a principal coordinate analysis (PcoA) to illustrate pairwise distances (Bray–Curtis) between pro- and eukaryotic soil communities. Bacterial community composition clearly separated between inoculation treatments along axis 1 and between different time points along axis 2. PERMANOVA analysis showed significant effects for treatment (Adonis $R^2 = 0.3$, $P = 0.001$), time point (Adonis $R^2 = 0.26$, $P = 0.001$), and their

Table 1 Balance of nitrogen inputs, uptake, and losses (a) and relative distribution of total N detected among plant uptake, nutrient losses, and remaining N in soil (b) at harvest from lysimeters inoculated with either a moderately simplified (MSC) or strongly simplified (SSC) soil community inoculum.

	MSC	SSC	% Change in SSC vs MSC
(a)			
Inputs			
Soil mineral N start	252 (11.57)	218 (14.37)	-13^{ns}
Fertilizer	60	60	–
Microbial biomass N start[a]	183 (24.53)	184 (22.36)	0^{ns}
Total input	495 (24.19)	462 (27.80)	-6.79^{ns}
Plant uptake			
Total shoot N	591 (17.62)	475 (15.97)	−19.6***
Total root N	17.7 (0.75)	13.8 (1.16)	−21.8*
Total uptake	608 (17.77)	489 (16.83)	−19.6***
N loss			
Gas			
N_2O–N	0.037 (0.01)	0.082 (0.01)	124*
N_2-gas	5.01 (0.98)	10.03 (1.17)	97*
Leachate			
NO_3–N leached	189 (7.98)	312 (16.05)	64.7***
NH_4–N leached	0.0014 (0.00)	0.0019 (0.00)	35.3^{ns}
NO_2–N leached	0.41 (0.33)	1.49 (0.33)	265^{ns}
Organic N leached	58.1 (7.80)	96.1 (8.84)	65.5**
Total loss	253 (12.85)	420 (23.25)	65.9***
Remaining			
Soil N pools at harvest			
Soil mineral N	51.7 (6.84)	73.1 (6.97)	41.2**
Microbial biomass N[a]	146 (9.25)	134 (5.98)	-8.29^{ns}
Total remaining	197 (12.51)	207 (12.45)	4.79^{ns}
Excess reactive N detected at harvest (total reactive N detected−inputs)	565 (34.40)	658 (34.57)	16.5^{ns}
(b)			
Reactive N detected			
Total reactive N detected in system (plant uptake + N loss + remaining N)	1058 (27.65)	1115 (23.51)	5.34^{ns}
Relative distribution of N in system (%)			
Uptake			
% of total detected, reactive N taken up by plants	58 (1.45)	44 (1.05)	***
Loss			
% of total detected reactive N lost	24 (0.90)	38 (1.05)	***
Remaining			
% of total detected reactive N remaining in soil at harvest	19 (0.98)	19 (0.72)	ns

Numbers in brackets indicate standard errors. Statistical differences between treatments are indicated as: ns, $P > 0.05$; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.01$. $n = 8$. All data given as kg ha^{-1} unless stated otherwise. Statistical tests have been performed using linear mixed-effects models. ns, not significant.

[a]Microbial biomass N was determined from frozen soil at start, while it was determined from fresh soil at harvest. Due to different storage conditions, both data may not be directly comparable.

Table 2 N use efficiency (unit plant N uptake per unit N input), N use inefficiency (N loss per unit N input), and net N efficiency (unit plant N uptake without directly corresponding N losses) for maize plants grown in lysimeters inoculated either with a moderately simplified (MSC) or strongly simplified (SSC) soil community inoculum.

	MSC	SSC	% Change in SSC vs MSC
N use efficiency (NUE)			
Plant N uptake per kg N input (kg ha^{-1})	1.97 (0.10)	1.81 (0.08)	-8.2^{ns}
N use inefficiency (NUI)			
N lost per kg N input (kg ha^{-1})	0.83 (0.07)	1.54 (0.13)	+86.6***
Net N efficiency (NNE = NUE − NUI)			
N uptake without direct environmental cost (kg ha^{-1})	1.14 (0.07)	0.27 (0.11)	−76.7***

Mineral soil N at the time of sowing and fertilizer inputs were considered as N input. Numbers in brackets indicate standard errors. Statistical differences between treatments are indicated as: ns, $P > 0.05$; ***, $P < 0.001$. $n = 8$. Statistical tests have been performed using linear mixed-effects models. ns, not significant.

interaction (Adonis $R^2 = 0.1$, $P = 0.005$; Fig. 4a). For eukaryotic communities, it also showed significant effects of time point (Adonis $R^2 = 0.16$, $P = 0.002$), treatment (Adonis $R^2 = 0.26$, $P = 0.001$), and their interaction (Adonis $R^2 = 0.13$, $P = 0.001$; Fig. 4b).

Differences in taxonomic composition of bacterial and eukaryotic relative ASV abundances were detected between treatments. The most abundant bacterial phyla detected in the dataset were Proteobacteria (MSC: 50.2% of sequences, SSC: 45.2%), Bacteroidota (MSC: 21.1%, SSC: 24.3%), Actinobacteriota (MSC: 7.5%, SSC: 8.3%), Gemmatimonadota (MSC: 5.7%, SSC: 7.9%), Acidobacteria (MSC: 4.8%, SSC: 3.2%), and Chloroflexi (MSC: 4.6%, SSC: 7.0%), see also Fig. S8.

For eukaryotic sequences, the most abundant phyla were Ascomycota (MSC: 39.5%, SSC: 31.6%), Ciliophora (MSC: 27.9%, SSC: 27.0%), Mortierellomycota (MSC: 22.7%, SSC: 35.6%), Chytridiomycota (MSC: 3.5%, SSC: 1.0%), and Nematoda (MSC: 2.5%, SSC: 0.4%), see also Fig. S9.

We calculated Shannon diversity (H') for prokaryotic and eukaryotic communities based on the normalized (relative abundance to total reads per sample) table with amplicon sequence variants (ASVs) at all five sampling time points. For prokaryotes, there was a significant interaction ($F_{4,24} = 9.609$, $P < 0.001$) between time point and treatment. Prokaryotic diversity was significantly higher in the MSC treatment compared with the SSC treatment at the time of sowing ($t_{1,5} = 9.148$, $P = 0.003$). At fertilization, microbial diversity in the MSC treatment dropped to levels comparable to the SSC treatment ($t_{1,5} = 1.76$, $P = 0.74$) but quickly recovered to similar levels than before fertilization, showing significant differences between treatments until harvest (Fig. 5a). Samples were collected after fertilizing and watering the lysimeters. Both seem to have strongly affected bacterial communities for a short period, only, since diversity levels were restored 120 h later.

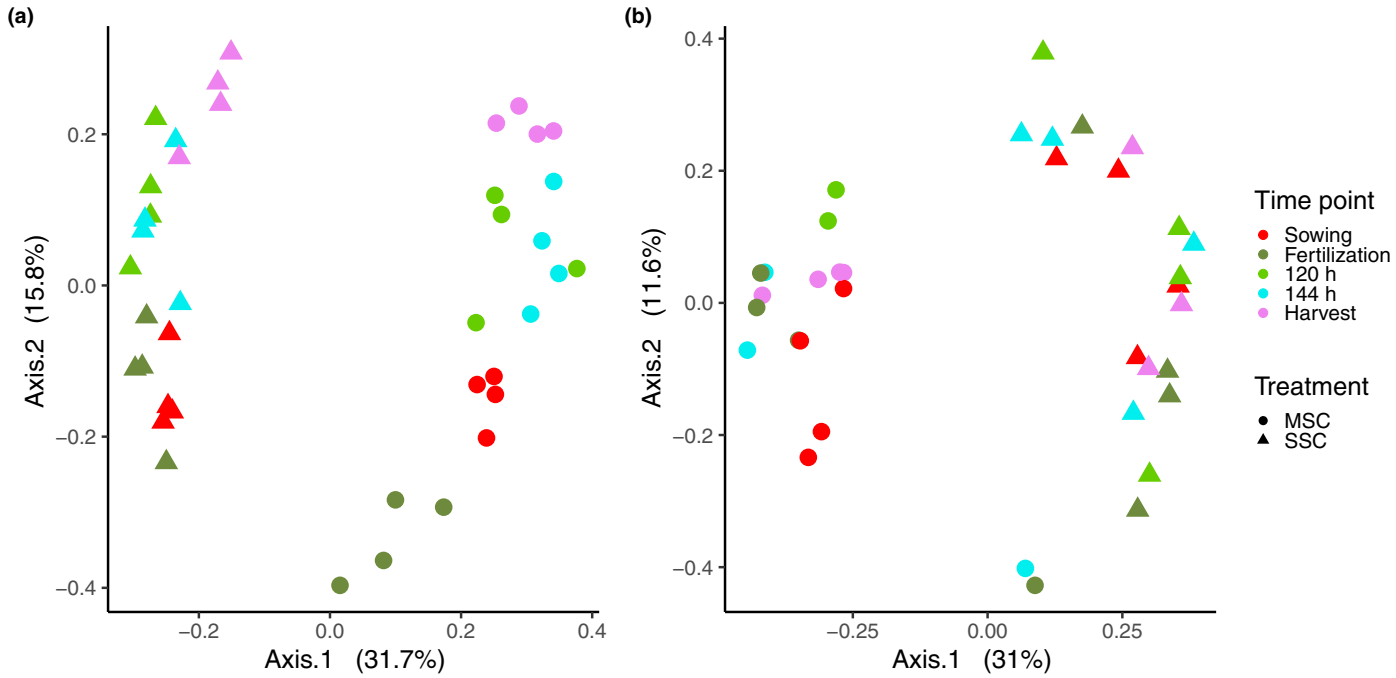

Fig. 4 Principal coordinate analysis (PcoA) performed on proportional sequence abundance derived from (a) 16S rRNA sequencing and (b) ITS2 sequencing illustrating differences between microbial communities in lysimeters inoculated either with a moderately simplified (MSC) or strongly simplified (SSC) soil community inoculum and sampled on five different time points during the study ($n = 4$).

For eukaryotic diversity, there was a significant treatment effect across all time points ($F_{1,5} = 45.7$, $P = 0.001$) and no significant effect of time point or interaction between both was observed. Eukaryotic diversity levels at sowing were clearly higher in the MSC compared with the SSC treatment but differences seemed to become smaller over time (Fig. 5b). Microbial evenness and observed richness of pro- and eukaryotic sequences are shown in Fig. S10.

Quantification of bacterial 16S gene copy numbers showed no significant differences between treatments. For ITS gene copy numbers, the SSC treatment showed a significant increase across all time points ($F_{1,29} = 12.14$, $P = 0.002$). However, pairwise comparisons for separate time points did not show any significant differences (Table S7).

We used shotgun metagenomics to reconstruct the N and P cycle and analyze the impact of the treatments on the respective enzyme coding genes at the time of fertilization and 120 h after fertilization, a time point when remarkable differences in N_2O and N_2 fluxes between both treatments were detected.

Differential abundance analysis revealed different N cycling gene abundances between treatments across both time points (Fig. 6). In the MSC treatment, we observed a significant ($P < 0.05$) increase in relative (compared with the SSC treatment) abundance of two genes involved in microbial ammonia assimilation (*glnA* and *GDH2*), one transporter gene involved in ammonia uptake (*amt*) and one archaeal (Phylum Thraumarchaeota) gene encoding for ammonia oxidase and one bacterial (Phylum Nitrospirae) gene encoding for nitrite oxidoreductase, both being involved in nitrification. Moreover, the nitric oxide reductase *norB*, involved in N_2O production during denitrification, was relatively more abundant in the MSC treatment, while the nitric oxide reductase *nirS* showed a slight increase at $P < 0.1$.

In the SSC treatment, we observed an increase ($P < 0.05$) in relative abundance of one gene encoding for a nitrate reductase involved in assimilatory nitrate reduction (*narB*), three ammonia monooxygenase subunits from the bacterial family Nitrosomonadaceae (*amoA, amoB*, and *amoC*) and the nitric oxide reductase *nirK* involved in N_2O production during denitrification. The nitrous oxide reductase *nosZ*, responsible for the formation of N_2, was slightly increased in the SSC treatment at $P < 0.1$.

For P transformation, differential abundance analysis revealed a higher ($P < 0.05$) relative abundance of four P transporter genes (*pstA, pstB, pstC*, and *pstS*) in the MSC treatment. In the SSC treatment, two genes involved in phosphonate and phosphinate metabolism (*phnX* and *PTERphp*), and three genes involved in organic phosphoester hydrolysis (*ugpQ, phoD*, and *aphA*) showed a significantly higher relative abundance (Fig. S11; Table S9).

Discussion

We manipulated soil biota communities, measured nutrient leaching and N_2O and N_2 emissions, and provide direct proof for the significant role of soil ecological processes in nutrient cycling. We observed 66% higher nitrogen losses and 20% lower plant N uptake in lysimeters with strongly simplified soil biota communities. This study provides a first assessment of the importance of soil biota communities for nutrient cycling, including direct and simultaneous quantification of nitrogen leaching losses and gaseous emissions, including N_2. Moreover, we assessed relationships between functional characteristics of soil biota

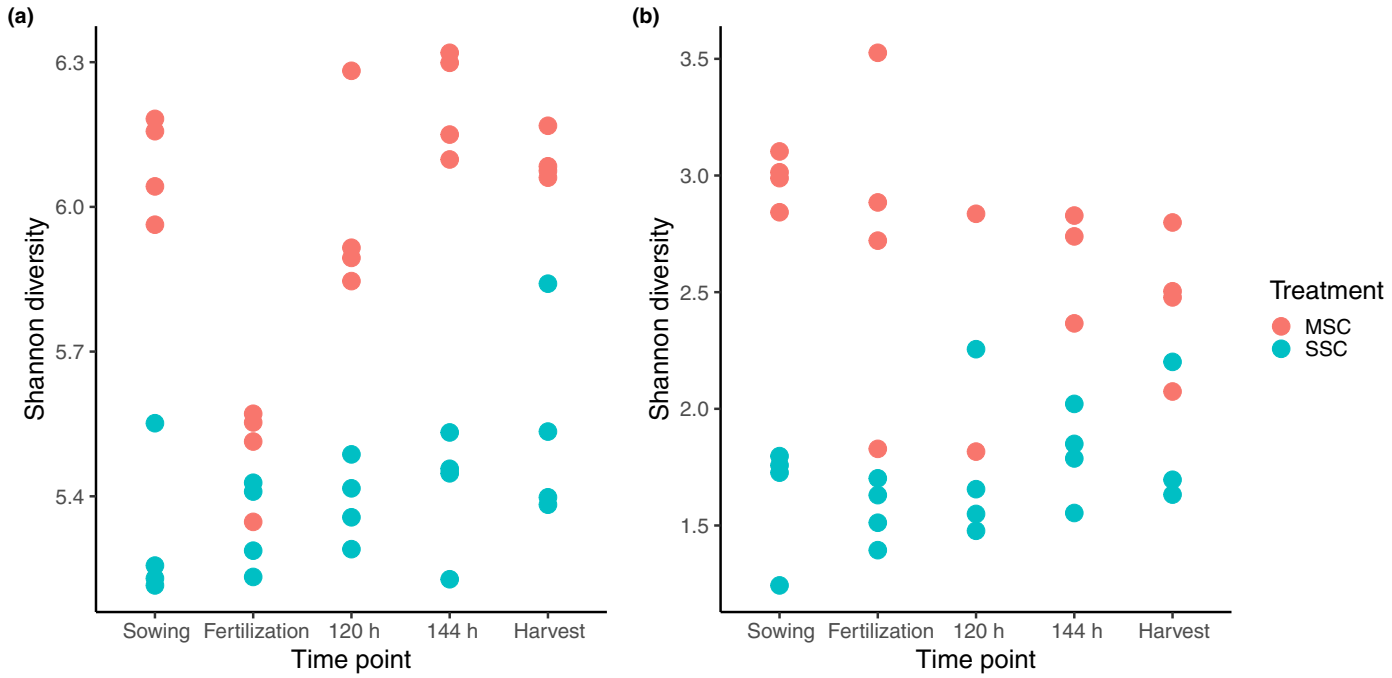

Fig. 5 Diversity levels (Shannon index) of soil microbial communities in lysimeters inoculated either with a moderately simplified (MSC) or strongly simplified (SSC) soil community inoculum sampled at five time points during the study. (a) Prokaryotic communities assessed through 16S metabarcoding, (b) eukaryotic communities assessed through ITS metabarcoding. Note that *y*-axes are truncated for improved presentation.

communities and N gas losses. Measuring N_2 emissions is inherently complicated (Knowles, 1982) and studies on the effects of soil biota or their communities on N_2 emissions are rare. Many studies focused on land-use and abiotic soil parameters as controlling factors (Stevens & Laughlin, 1998; Thomas *et al.*, 2019) and few related direct manipulations of specific groups of soil microorganisms to N_2O emissions in microcosms (Philippot *et al.*, 2011; Lubbers *et al.*, 2013; Bender *et al.*, 2014; Lazcano *et al.*, 2014; Storer *et al.*, 2017; Li *et al.*, 2023). Insights into the effects of direct manipulations of soil biota communities on the resulting emissions of N_2O and N_2, simultaneously, had hitherto been missing. Our study shows that soil biota communities can play a substantial role in N cycling and that differences in their abundance and diversity regulate productivity, nutrient use efficiency, and environmental impact of plant–soil systems. We provide proof of concept that the composition of soil biota communities is a major determinant of sustainable N cycling.

The distribution of nutrients in our model system differed strongly between the two soil biological treatments. Plant N uptake was 20% lower in the strongly simplified soil community, and plant P uptake was reduced by 58% compared with the moderately simplified soil community treatment. These numbers show that the ability of plants to take up nutrients is strongly affected by the composition of the biological community present in soil. Moreover, we found gaseous N emissions to be doubled (+101%) and N leaching losses to be increased by 65% in the strongly simplified soil community treatment, showing that soil biology strongly affects the ability of plant–soil systems to retain nutrients. Interestingly, the nitrogen use efficiency, reflecting plant N uptake per unit available N at the start of the experiment, was only slightly lower in the strongly simplified soil community treatment. However, the net N efficiency, representing the amount of plant N uptake minus corresponding N losses to the environment per unit available N at the start of the experiment, was reduced by 76% in the strongly simplified soil community treatment (Table 2). These pronounced effects of soil biota on N use efficiency could potentially have substantial ecological and economic implications. The high N fertilizer inefficiency reported globally with up to 50% of N fertilizer being unused by crops (Liu *et al.*, 2010) might be partly caused by highly disturbed soil biological communities.

Soil available P levels at the start of the experiment were characterized as sufficient for plant growth in both treatments (Flisch *et al.*, 2009). Still, plant P uptake was substantially higher in the moderately simplified soil community treatment showing higher microbial diversity and abundance of AMF. These results suggest that the composition of soil biota communities is a crucial factor to consider when aiming at optimizing P use efficiency. The clearest difference in soil biological characteristics between both treatments was the presence of AMF. The ability of AMF to support plant P nutrition is well known (Smith & Smith, 2011; van der Heijden *et al.*, 2015). It appears well possible that AMF are also responsible for the substantial differences in plant P uptake between both treatments. However, soil metagenomic analysis showed several bacterial genes involved in P uptake and immobilization being relatively more abundant in the moderately simplified soil community treatment (as compared to the strongly simplified soil community treatment treatment), suggesting that microbial processes other than AMF activity also contributed to these effects. In contrast to N leaching, P leaching, although

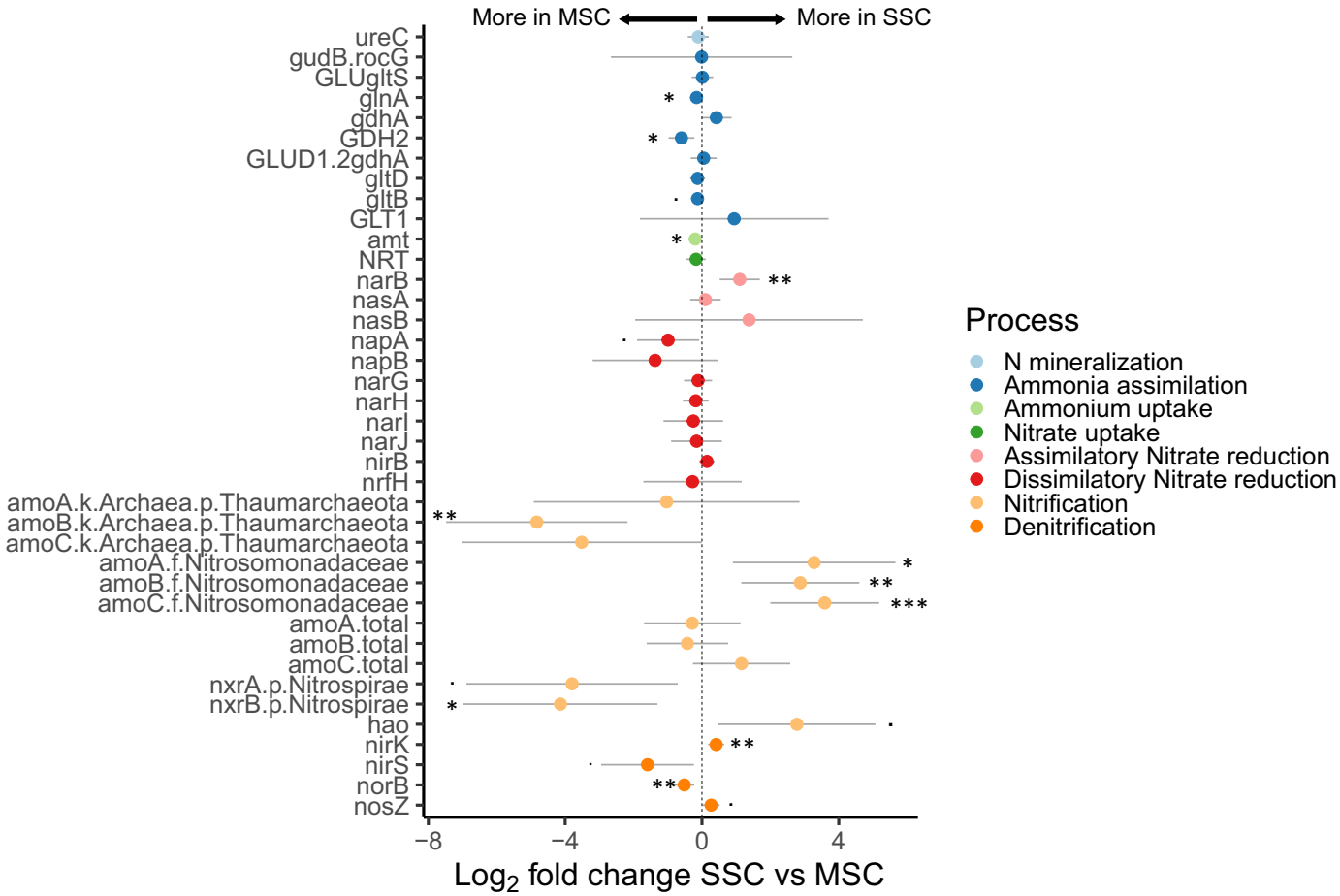

Fig. 6 Log_2 fold change in TPM (transcripts per kilobase million) abundance of enzymes involved in N cycling processes in lysimeters inoculated with a strongly simplified soil community (SSC) compared with lysimeters inoculated with a moderately simplified soil community (MSC) across two time points during maize growth. Enzymes were inferred from functional annotation of soil metagenomic sequences against GenBank nr NCBI, eggNOG, KEGG, and PFAM databases (see the Materials and Methods section for details). See Supporting Information Table S8 for the full names of the enzymes and an overview of the KEGG numbers assigned to the different genes shown. Datapoints on the right-hand side of the line are relatively more abundant in the SSC treatment, and those on the left-hand side are more abundant in the MSC treatment. Significance tests were performed using differential abundance analysis in the R package DESeq2 (Love *et al*., 2014). Significance levels: ***, $P < 0.001$; **, $P < 0.01$; *, $P < 0.05$; •, $P < 0.1$. Error bars represent 95% confidence intervals ($n = 4$).

quantitatively much lower, was higher in the treatment with moderately simplified communities. It is possible that increased microbial activity in that treatment led to higher release of enzymes or organic acids promoting decomposition and the export of soluble P from soil (Tran *et al.*, 2020).

Even though soil provided ample amounts of N in both treatments, moderately simplified soil communities induced much more efficient N transfer from soil to plant as compared to strongly simplified communities, where substantially more N was lost to the environment. The relative increase in abundance of three genes involved in ammonia uptake and assimilation in the moderately simplified soil community treatment (Fig. 6), suggests an increased N uptake capacity of that system and providing one explanation for the reduced N losses. Moreover, pot experiments have shown that AMF can reduce N losses from soil through leaching and as N_2O and can enhance plant N nutrition (van der Heijden, 2010; Asghari & Cavagnaro, 2012; Bender & van der Heijden, 2015; Cavagnaro *et al.*, 2015; Bowles *et al.*, 2018; Qiu *et al.*, 2021). It remains unclear whether, and to what extent, such effects are caused directly by increased N uptake through AMF, indirectly through AMF effects on microbial communities involved in N cycling, or a combination of both (de Vries *et al.*, 2013; Zhang *et al.*, 2016; Zhao *et al.*, 2021). Recently, the importance of synergistic interactions between AMF and other soil microorganisms for increasing plant N acquisition and N cycling has been highlighted (Hestrin *et al.*, 2019; Li *et al.*, 2023).

Another possible explanation for the observed effects on N cycling is that the strong increase in plant P uptake in the moderately simplified soil community treatment led to subsequent increases in crop N uptake to maintain stoichiometric nutrient ratios. Plant N : P ratios were indicative for N limitation in both treatments (Koerselman & Meuleman, 1996; Güsewell, 2004) and were significantly lower in the moderately simplified soil community treatment compared with the strongly simplified soil community treatment, supporting this theory. A resulting

strong plant N-sink could, therefore, have contributed to the reduced nutrient losses observed in the moderately simplified soil community treatment. Our results indicate that soil biota-induced differences in P uptake could, potentially, translate into massive effects on N cycling, pointing to the importance of nutrient stoichiometry (C : N : P ratio) for the functional consequences of soil biota activity (Griffiths *et al.*, 2012).

We observed significantly lower bacterial and eukaryotic diversity in the strongly simplified soil community treatment compared with the moderately simplified soil community treatment (Fig. 5). It has been shown that reductions in microbial diversity can lead to a decline in specific soil processes (Philippot *et al.*, 2013) and also to a decline in multiple ecosystem functions simultaneously, including the ability to retain nutrients in the plant–soil system (Wagg *et al.*, 2014, 2019). Higher microbial diversity could lead to enhanced resource use complementarity and synergistic interactions among different organisms and therefore enhanced plant productivity and more closed resource cycles (Van Der Heijden *et al.*, 2016; Morriën *et al.*, 2017), providing an additional, possible explanation for the observed effects in our study.

The vast majority of N leaching losses occurred in the form of nitrate. Nitrification, the microbial oxidation of ammonium to nitrate, is the major nitrate-producing process in soil. We observed a relative increase in three important nitrification genes (*amoA, amoB*, and *amoC*), in the strongly simplified soil community treatment (Fig. 6), suggesting that higher nitrification process rates may have contributed to the high nitrate leaching losses in this treatment. While the three ammonia oxidase genes relatively more abundant in the strongly simplified soil community treatment could be assigned to the bacterial family Nitrosomonadacea, the moderately simplified soil community treatment showed a relative increase in one ammonia oxidase subgroup from the archaea phylum Thaumarchaeota. This suggests that ammonia oxidation was performed by taxonomically different communities in both treatments and points to the importance of microbial community composition for nitrogen cycling processes.

The process of nitrification can also produce substantial amounts of N_2O (Van Groenigen *et al.*, 2015). Moreover, nitrate forms the direct basis for the process of denitrification. Therefore, increased rates of nitrification and higher availability of nitrate both provide potential explanations for the increase in N_2O and N_2 emissions observed in the strongly simplified soil community treatment. The nitrate reductase *nirK*, a key enzyme for denitrification, showed significantly higher relative abundance in the strongly simplified soil community treatment, providing a direct mechanistic explanation for the higher N_2O emissions in this treatment. However, the nitrite reductase gene *nirS* tended to be relatively more abundant in the moderately simplified soil community treatment, although this effect being just not significant ($P < 0.1$). Both genes are evolutionarily unrelated and represent two ecologically distinct denitrifying groups (Sun & Jiang, 2022), indicating that the composition of denitrifying communities differed among treatments and that *nirK*-driven N_2O production caused the higher emissions observed in the strongly simplified soil community treatment. Interestingly, the nitric oxide reductase *norB* showed significantly higher relative abundance in the moderately simplified soil community treatment, although this treatment had significantly lower N_2O emissions. This gene is responsible for the second step in N_2O production converting nitric oxide produced by the nitrite reductases into N_2O Philippot (2002). Nitrous oxide reductase (*nosZ*), the main gene consuming N_2O and transforming it into N_2, tended to be relatively more abundant in the strongly simplified soil community treatment, although the effect just being not significant ($P < 0.1$). This points toward a relatively higher abundance of *nosZ*-containing denitrifiers in the strongly simplified soil community treatment causing the increased N_2 emissions in this treatment. Taken together, the soil metagenomic analyses in both treatments reveal patterns that partly coincide with the observed effects on nitrogen losses – strongly increased nitrate leaching, as well as gaseous N_2O and N_2 emissions in the strongly simplified soil community treatment – and provide insight into the soil functional processes underlying them. It is important to note that the functional interpretations of metagenome data are based on abundances relative to the total number of sequences in each treatment. A higher relative abundance of a particular gene in one treatment compared with another does not automatically indicate an absolute increase in a particular function. The data, therefore, can only be used as an indicator for potential mechanisms behind observed nutrient dynamics. It is, however, noteworthy that soil microbial biomass C did not show significant differences between treatments at the start of the experiment (Table S3).

Agricultural management imposes multiple stresses on soil biota that have been repeatedly shown to reduce their abundance and diversity. Information on the functional implications of such adverse effects on soil communities is rare and often lacks the explicit quantification of nutrient cycling processes (e.g. Rillig *et al.*, 2023). In earlier work with a greenhouse model-system study, we showed that reductions in soil microbial diversity and community composition lead to an overall decline in ecosystem functioning and nutrient cycling (Wagg *et al.*, 2014, 2019). Here, we show on a larger and ecologically more relevant scale that such effects could potentially have severe consequences for global crop production, the pollution of waterways, greenhouse gas emissions, and overall N use efficiency. Changes in soil food webs, such as the loss of specific organism groups, reductions in diversity, or the composition of microbial communities can compromise internal ecosystem processes that retain N in the plant–soil system and contribute to sustainable nutrient cycling.

Although our results cannot be directly transferred to field situations because the work was done in lysimeters filled with sterilized soil and simplified soil communities, they highlight the potential of soil biota to enhance the sustainability of crop production. A range of studies have shown that very intensive agricultural management can lead to the simplification of soil biota communities and can have a strong, negative impact on organisms such as earthworms or arbuscular mycorrhizal fungi (Helgason *et al.*, 1998; Verbruggen *et al.*, 2010; Tsiafouli *et al.*, 2015;

Edlinger *et al.*, 2022). To study the impact of agricultural intensification on nutrient cycling, further studies should collect soils from such very intensively managed agricultural fields and compare their nutrient cycling dynamics to less intensively managed soils. In line with our findings, a recent study demonstrated that the nutrient use efficiency of applied nutrients was linked to soil health and was higher in organically managed compared with conventionally managed soils (Toda *et al.*, 2023). The model crop maize has a high N demand and may, therefore, be a drastic showcase. Results might differ using other, less N-demanding crops. Knowledge of detailed organism–function relationships needs to be integrated with information on biotic interactions in complex food webs and on biogeochemical process measurements to increase our understanding of the mechanisms driving plant nutrient uptake and nutrient loss. While our soil functional analyses are DNA-based, RNA-based data may provide a more real-time picture of soil nutrient cycling processes, potentially leading to improved alignment of genetic and process measurement data on nutrient cycling processes.

There is an urgent need to enhance the sustainability of agricultural production. Our study shows that soil biological communities play a major role in achieving this goal. Future research should focus on intensified 'soil ecological engineering' (Bender *et al.*, 2016) to develop management strategies that promote soil biota to maximize their contribution to agricultural production, reduce fertilizer applications and to increase overall agricultural sustainability.

Acknowledgements

The authors would like to thank Philipp Weber and Toni Hirscher for their help in setting up the experiment, Alain Held for support at harvest, Rachael Carolan, Caroline Scherrer, Martin Zuber, Diane Bürge, and Agush Loshi for their valuable contributions to sample analyses, Aman Gill and Karen McGeough for helpful discussions on data analyses and Sylvia Gschwendtner for data processing and uploading to the online repository. This work was funded by the Swiss National Science Foundation (grant 31003A-166079 awarded to MGAH). Open access funding provided by Agroscope.

Competing interests

None declared.

Author contributions

SFB and MGAH designed the experiment. SFB performed the research. MS, SS, SK, CJW, RJL and JR provided new methods/analytical tools. RM-C, KP-Z, GV, KH, EP and SFB analyzed the data. SFB and MGAH wrote the paper and all authors commented on the manuscript.

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Data availability

The data that support the findings of this study are available in the Supporting Information of this article. Sequencing raw data from this study are available at the sequence read archive (http://www.ncbi.nlm.nih.gov/sra) at the National Center for Biotechnology Information under accession no. PRJNA648050.

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Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Dataset S1 Data generated and analyzed in this study.

Fig. S1 Schematic overview of inoculum production.

Fig. S2 Diversity levels and taxonomic composition of soil microbial communities in the moderately simplified or strongly simplified soil community inocula.

Fig. S3 Overview of the Lysimeter system used in this study.

Fig. S4 Schematic overview of the timeline of the study phases, experimental activities, and sampling events.

Fig. S5 Correlation between N_2O–N fluxes determined by GC-IRMS and N_2O–N fluxes as determined by continuous-flow analysis.

Fig. S6 Rarefaction curves of 16SrRNA and ITS sequences for all soil samples.

Fig. S7 Nonpareil curves of soil metagenomic sequences from 16 soil samples collected from lysimeters from the MSC and SSC treatments at two different time points.

Fig. S8 Relative abundance of bacterial genera as determined by 16S rRNA sequencing in lysimeters inoculated either with a

moderately simplified or strongly simplified soil community inoculum at five time points during the experiment.

Fig. S9 Relative abundance of eukaryotic genera as determined by ITS2 sequencing in lysimeters inoculated either with a moderately simplified or strongly simplified soil community inoculum at five time points during the experiment.

Fig. S10 ASV richness and evenness indices of microbial communities in lysimeters inoculated either with a moderately simplified or strongly simplified soil community inoculum assessed at five time points during the experiment.

Fig. S11 Log_2 fold change in TPM (transcripts per kilobase million) abundance of enzymes involved in P cycling processes in lysimeters inoculated with a strongly simplified soil community treatment compared with lysimeters inoculated with a moderately simplified soil community treatment across two time points during maize growth.

Methods S1 Additional methodological details not included in the main text.

Table S1 Nutrient solution applied during inoculum production.

Table S2 Biological analyses of inocula used in the study.

Table S3 Soil parameters in Lysimeters inoculated with two different biological inocula at the time of maize sowing.

Table S4 Results of statistical tests assessing the effects of soil biological inoculum on maize yield and nutrient exports through plant uptake, leaching losses, and as gaseous emissions from lysimeters inoculated with either a moderately simplified or strongly simplified soil community inoculum.

Table S5 Results of statistical tests assessing the effects of soil biological inoculum on soil nutrient pools and nutrient efficiency calculations from lysimeters inoculated with either a moderately simplified or strongly simplified soil community inoculum.

Table S6 Average numbers of Collembola (springtails) and Acari (mites) per 200 cm^3 sample taken from 0 to 10 cm of soil per lysimeter at the end of the experiment and average number of nematodes detected in 100 g of dry soil per lysimeter at the end of the experiment.

Table S7 Quantification of 16S rRNA and ITS gene copy numbers across all time points in soils inoculated with either a moderately simplified or strongly simplified soil community inoculum.

Table S8 Abbreviation, full name, corresponding KEGG, PFAM or eggNOG number, and N transformation process of the enzymes presented in Fig. 5.

Table S9 Abbreviation, full name, corresponding KEGG or PFAM number, and P transformation process of the enzymes presented in Fig. S11.

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