Soil Biology and Biochemistry 203 (2025) 109737

Contents lists available at ScienceDirect

Soil Biology and Biochemistry

journal homepage: www.elsevier.com/locate/soilbio

Organic cropping systems alter metabolic potential and carbon, nitrogen and phosphorus cycling capacity of soil microbial communities

Hans-Martin Krause [a,*], Ralf C. Mueller [a], Martina Lori [a], Jochen Mayer [b], Paul Mäder [a], Martin Hartmann [c]

[a] Department of Soil Sciences, Research Institute of Organic Agriculture (FiBL), 5070 Frick, Switzerland
[b] Agroecology and Environment, Agroscope, 8046 Zürich, Switzerland
[c] Department of Environmental Systems Science, ETH Zürich, 8092 Zürich, Switzerland

ARTICLE INFO

Keywords:
Long-term experiment
System comparison
Soil functional diversity
Shotgun metagenome

ABSTRACT

Intensive agriculture can impair soil quality and threaten the provision of critical soil ecosystem services. Organic cropping systems aim to ensure sustainable production by promoting soil biodiversity to enhance soil functioning and regulate nutrient cycling through microbial processes. While taxonomic changes in microbial community composition in response to agricultural management are well described, there is still a fundamental knowledge gap when it comes to the impact of cropping system on soil functional diversity. Therefore, we revisited the 42-year-old DOK field experiment and used shotgun metagenomics to assess the metabolic potential and nutrient cycling capacities in organic and conventionally managed soils. The functional annotation of 11.4 billion reads to universal (EC, SEED), as well as carbon (CAZy), nitrogen (NCycDB) and phosphorus (PCycDB) cycling gene ontologies showed that manure fertilization was the main factor altering soil metabolic potential. But also, organic management practices, such as omission of synthetic pesticides and mineral fertilization induced changes in soil metabolic potential e.g. by enriching functional genes involved in organic phosphorus acquisition, nitrate transformation, organic degradation and non-hydrolytic carbohydrate cleavage. Conventional systems, receiving mineral fertilization and chemical plant protection, enriched genes associated with inorganic nutrient acquisition and transcriptional activity. The results of this study demonstrate that cropping systems influence the functional potential of soils, affecting fundamental mechanisms of nutrient cycling and thus soil regulating capacity. Consequently, cropping systems can be utilized to steer the regulating potential of agricultural soils and to lower the environmental impact of food systems.

1. Introduction

Soil is a complex ecosystem essential for agricultural production. In recent decades, the intensification of agricultural practices to improve food production has resulted in the increased use of agrochemicals and demand for land (Zhang et al., 2021; Potapov et al., 2022). This has led to several detrimental environmental impacts, including biodiversity loss, natural ecosystem eutrophication, higher greenhouse gas emissions and the degradation of soil quality (Foley et al., 2005; Tilman et al., 2011; IPBES, 2019; IPCC, 2023). The primary challenge for the agricultural sector is to ensure the security of food supplies for a growing population while reducing the environmental impact of food production. As agricultural soils provide vital ecosystem services, the proper stewardship of soil, which entails the maintenance and harnessing of soil functions, represents a cornerstone of sustainable agriculture (Adhikari and Hartemink, 2016). While external inputs may compensate for soil provisioning services, soil regulating and supporting services mainly rely on biological processes that are intrinsically linked to the concept of soil quality and soil health (Bünemann et al., 2018; Lehmann et al., 2020).

Due to its focus on soil biodiversity and mitigation of environmental impacts, organic cropping systems have gained increasing interest as a viable alternative to high-input conventional farming (Mäder et al., 2002; Seufert and Ramankutty, 2017), despite lower yields (Seufert et al., 2012; Knapp and van der Heijden, 2018; de la Cruz et al., 2023). Since organic cropping renounces the use of mineral fertilizers and synthetic plant protection inputs, nutrient supplies are based on systemic approaches such as integrating livestock and utilizing biological

* Corresponding author. author
E-mail address: hans-martin.krause@fibl.org (H.-M. Krause).

https://doi.org/10.1016/j.soilbio.2025.109737
Received 12 October 2024; Received in revised form 27 January 2025; Accepted 1 February 2025
Available online 4 February 2025

nitrogen fixation through legumes. Yet, covering crop demand for phosphorus remains a challenge, especially for organic cropping systems that rely on organic phosphorus sources and thus microbial phosphorus solubilization in soils (Oberson and Frossard, 2015; Oberson and Frossard, 2015; Oberson and Frossard, 2015; Oberson and Frossard, 2015).

Long-term field experiments are the backbone of agricultural research since they deliver crucial insights in agronomic production and environmental impacts of cropping systems. The DOK experiment (bio**D**ynamic, bio**O**rganic and **K**onventionell (German for conventional) is the world's oldest field experiment comparing organic and conventional cropping in a system comparison approach since 1978 (Mäder et al., 2002). Following a system approach, inputs of organic matter and nitrogen differ between systems. Highest fertilizer inputs in the mixed conventional system did not significantly change soil organic carbon contents or soil nitrogen stocks over 42 years (Krause et al., 2022; Oberson et al., 2024). Conversely, organic cropping systems demonstrated the capacity to maintain or even enhance soil carbon contents and nitrogen stocks despite 15% and 45% lower average organic matter and nitrogen inputs in organic systems compared to conventional systems, respectively (Krause et al., 2022; Oberson et al., 2024). Given that differences in soil carbon and nitrogen contents cannot be attributed to the quantity of organic matter inputs alone, it was hypothesized that microbial capacity to cycle carbon in the various cropping systems represents a significant factor influencing long-term trends in soil organic carbon contents and soil organic matter fractions (Mayer et al., 2022). In addition, unexpectedly high nitrogen use efficiencies were observed in the cropping systems of the DOK experiment, which suggests that most soil nitrogen becomes plant-available over the long term (Oberson et al., 2024). In organic cropping systems, the microbial capacity to mineralize organically bound nitrogen is vital to ensure crop nutrition due to the absence of mineral fertilizers. The meeting of crop phosphorus requirements in organic systems also relies on microbial activity. While soil phosphorus stocks did not differ between organic and conventional systems after 42 years, the 36% reduction in phosphorus inputs in organic systems and the consistently negative phosphorus balances observed in all systems receiving organic fertilizer (Krause et al., 2024), reinforce the dependence of organic cropping systems on microbial capacity for phosphorus mobilization. These observations call for an in-depth analysis of the functional potential of soils for nutrient cycling in response to long-term organic cropping practices.

It is commonly acknowledged that agricultural management causes structural and chemical changes in the soil, ultimately impacting microbial habitats and diversity (Hartmann and Six, 2023). Indeed, all cropping systems in the DOK experiment show distinct bacterial and fungal communities and the amendment of farmyard manure was identified as main driver of these differences (Hartmann et al., 2015), next to fertilization intensity (Lori et al., 2023). Yet, there is a fundamental knowledge gap when it comes to linking microbial taxonomic diversity with community functioning (Louca et al., 2018). The fact that taxonomically distinct organisms can share similar metabolic pathways calls for a gene-centric approach to disentangle the effects of the cropping system on the metabolic potential of the soil microbiome, which is dominated by uncultivated and undescribed taxa (Banerjee and van der Heijden, 2023). Various ontologies have been developed for classifying functional genes, for example, based on enzymatic reactions (EC (Bairoch, 2000);) or hand-curated subsystems (SEED (Overbeek et al., 2014);). More recently, specific databases focusing on genes encoding for carbohydrate active enzymes (CAZy (Drula et al., 2022);), nitrogen cycling (NCycDB (Tu et al., 2019);) and phosphorus cycling (PCycDB (Zeng et al., 2022);), facilitate the targeting of regulatory capacities for nutrient cycling in agriculturally managed soils.

Therefore, this study revisited the DOK long-term system comparison field experiment to i) assess the influence of organic and conventional cropping systems on the soil metabolic potential and nutrient cycling capacity by comparing compositional differences in functional genes and ii) identify functional genes and gene classes associated with specific cropping systems to better understand long-term changes in carbon, nitrogen and phosphorus stocks. We hypothesized that the inputs of complex organic matter in organic and integrated conventional cropping systems will modify carbon, nitrogen and phosphorus cycling capacities by increasing the abundance of functional genes associated with the degradation of complex compounds compared to purely minerally fertilized or unfertilized systems.

2. Materials and methods

2.1. Field experiment

The DOK long-term field experiment is located in Therwil, Switzerland (47°30′N; 7°32′E; 306 m above sea level) on a haplic luvisol with 15 % sand, 70 % silt and 15 % clay. The climate is mild with a mean annual temperature of 10.5 °C and mean annual precipitation of 842 mm. The field experiment was established in 1978 and compares two organic – one bioorganic (BIOORG) and one biodynamic (BIODYN) – with two conventional systems – a mixed conventional (CONFYM) and a mineral conventional (CONMIN) – and an unfertilized control (NOFERT). The CONFYM system mimics integrated production systems with farmyard manure and mineral fertilizer, while the CONMIN system is purely based on mineral fertilizers without any organic inputs (Table 1). All systems follow a seven-year crop rotation with maize followed by green manure, soy, winter wheat followed by green manure, potatoes, winter wheat and two years of grassclover. The field plots (5 × 20 m) are arranged as a randomized split–block design with four replicates of each treatment and crop, and three temporally shifted parallels (Fig. S1). The systems BIODYN, BIOORG and CONFYM mimic mixed arable-livestock systems, and receive farmyard manure corresponding to 1.4 LU (livestock units) per hectare. Similar crop rotations and stocking densities in organic and conventional systems are rooted in typical farm structure of the region with a high share of mixed arable-livestock systems. Manure inputs in these systems differ in terms of manure processing strategy with increasing storage time and aeration effort from CONFYM < BIOORG < BIODYN. Briefly, CONFYM receives stacked manure, BIOORG rotten manure, and BIODYN composted manure. CONMIN receives exclusively mineral fertilizers and might better represent conventional intensive agriculture than CONFYM at a global scale. Organic fertilization in CONFYM is complemented with mineral nitrogen, phosphorus and potassium inputs according to Swiss fertilization recommendations (Table 1). As manure nutrients are assumed to be only partly plant-available, CONFYM receives the highest total inputs. (Table 1). Quantities of organic matter, nitrogen and phosphorus inputs via manures, slurries and mineral sources are listed in Supplementary Table S1. Plant protection in BIOORG and BIODYN is mainly done mechanically except from copper-sulphate application during potato cultivation in BIOORG, and the use of *Bacillus thuringiensis* subsp. *Tenebrionis* against Colorado beetles (*Leptinotarsa decemlineata*). In the CONFYM and CONMIN, synthetic fungicides, herbicides and insecticides are applied if the economic thresholds are surpassed to comply with the regional standard of integrated production. The BIODYN system additionally receives biodynamic preparations, while growth regulators are used for wheat in CONMIN and CONFYM. Consequently, the organic systems, BIOORG and BIODYN differ in manure processing and the additional use of copper sulphate in BIOORG and biodynamic preparations in BIODYN. The conventional systems CONFYM and CONMIN differ mainly in the fertilization strategy, with mineral fertilization only in CONMIN and organic fertilization supplemented with mineral sources in CONFYM. Details on the implementation and management of the DOK experiment can be reviewed in Krause et al. (2020).

2.2. Soil sampling

Soil sampling was conducted in spring 2019 during winter wheat

Table 1
Management details of the DOK cropping systems and mean annual inputs (across crop rotation period 2–6) of organic matter, total and mineral nitrogen, phosphorus and potassium.

Farming system	NOFERT	BIODYN	BIOORG	CONFYM	CONMIN
Plant protection					
Weeds	mechanical weeding			mechanical weeding and herbicides	
Diseases	indirect		indirect, copper to potatoes	fungicides	
Pests	bio-control, plant extracts, preventive measures			insecticides and preventive measures	
Special treatments	biodynamic preparations		–	plant growth regulators	
Mean annual plant protection inputs (kg/ha * yr)					
active ingredient equivalents	–	0.09	0.33	2.83	2.83
Fertilizer type					
Organic inputs	–	composted farmyard manure, slurry	rotted farmyard manure, slurry	stacked farmyard manure, slurry	–
External inputs	–	rock dust	rock dust, K and Mg	mineral NPK fertilizer	
Mean annual fertilizer inputs (kg/ha * yr)					
Organic matter	–	1911	2032	2314	–
Total nitrogen	–	93	96	171	121
Mineral nitrogen	–	26	30	113	121
Phosphorus	–	24	24	37	38

cultivation after the sixth crop rotation period. Bulk soil samples were taken to a depth of 20 cm as composite samples (12 cores per field plot of 5 m × 20 m) from 4 replicated plots per treatment in subplot C (Fig. S1). Sampling was performed three times with T1 on February 26, T2 on April 8 and T3 on April 15. The first samples were taken before the vegetation period, after the winter rest. Subsequently, the second and third sampling dates were scheduled to capture the first fertilization during winter wheat cultivation (sown on October 11, 2018). Homogenized soil samples were immediately transported to the laboratory in cooling boxes, sieved to 5 mm and stored at −20 °C. Fertilization of winter wheat with calcium-ammonium-nitrate (CONMIN; 70 and 30 kg N ha^{-1}, CONFYM 40 and 30 kg N ha^{-1}) and slurry (BIOORG 35 and 20 m^3 ha^{-1}, BIODYN 25 and 23 m^3 ha^{-1}) was performed on March 13 and April 9.

2.3. DNA extraction and sequencing

Soil samples were lyophilized and a subsample of 400 mg was used for DNA extraction using the NucleoSpin 96 Soil kit (Machery-Nagel, Dürren, Germany) with the SL2 + Enhancer SX lysis buffer according to the manufacturer's instructions. DNA extraction was done in technical duplicates and pooled after extraction, yielding a total of 60 samples (5 cropping systems x 4 field replicates x 3 time points). Library preparation and paired-end sequencing (PE150) on an Illumina NovaSeq 6000 was done at the Génome Québec Innovation Centre (Montreal, Canada) according to the sequencing guidelines provided by Illumina and yielded 11, 448, 283,174 individual reads with a total of 1729 Gigabases. Metadata and raw sequences have been deposited in NCBI's Sequence Read Archive (PRJNA924160).

2.4. Sequencing data processing

All 60 samples were processed individually. First, the raw sequence data was quality checked with FastQC v0.11.9 (Andrews, 2012), and Illumina adapters and poly-G/N tails trimmed with FASTP v0.22.0 (Chen et al., 2018). Low-complexity reads were filtered with PRINSEQ v0.20.4 (Schmieder and Edwards, 2011) and phi X 174 removed with USEARCH v9.2.64 (Edgar, 2010). The remaining reads were then quality filtered using VSEARCH v2.21.1 (Rognes et al., 2016), and dereplicated with idseq-dedup v0.1.0 (Zuckerberg, 2022). DNA sequencing yielded 1.73 Tb raw data in total of which 1.35 Tb remained after pre-processing. The pre-processed reads were assembled into contigs with minimum contig length 200 bp using MEGAHIT v1.2.9 (Li et al., 2015). Putative open reading frames were inferred by Prodigal v2.6.3 (Hyatt et al., 2010), and reported as protein-coding nucleotide sequences and translated into amino acid sequences. Then, pre-processed reads were mapped to putatively protein-coding genes (nucleotide sequences) with Bowtie2 v2.4.1 (Langmead and Salzberg, 2012) and the mapping statistics extracted using Samtools v1.15 (Li et al., 2009; Danecek et al., 2021). The mapping statistics were appended to the fasta headers of the amino acid sequences of predicted genes, which were subsequently aligned to NCBI's non-redundant (nr) database of protein-coding genes (O'Leary et al., 2016; Agarwala et al., 2018) (download: April 26, 2022) using DIAMOND v2.0.15 (Buchfink et al., 2021) with the expect value set to 10^{-5} and taxon search space restricted to archaea and bacteria. 168 Gb contributed to contig assembly with a mean contig N50 of 464 bp (minimum contig length cut-off set to 200 bp). Within these contigs, 422 million genes were predicted with a total length of 151 Gb to which pre-processed reads were mapped with Bowtie2. DIAMOND could align 360 Mio genes to the nr database. Hits were annotated with the tool daa-meganizer from the Community Edition of MEGAN v6.23.0 and MEGAN map Feb2022 (Huson et al., 2016), which includes mappings to the classification systems EC (Gough et al., 2001) and SEED (Overbeek et al., 2014), among others. Of these, a total of 125 Mio genes could be assigned to 4229 distinct terms (or accession numbers) in EC and 101 Mio genes assigned to 789 terms in SEED. Genes involved in carbohydrate, nitrogen and phosphorus metabolism were annotated more explicitly using resources from CAZy/dbCAN (Huang et al., 2018; Drula et al., 2022), NCycDB (Tu et al., 2019) and PCycDB (Zeng et al., 2022), respectively. In brief, CAZy (20220608), NCycDB (100_2019Jul) and PCycDB (20220317) sequence data was downloaded and prepared for DIAMOND alignments. Predicted genes were then aligned to these references using DIAMOND with the expect value set to 10^{-5}. Mapping statistics were extracted from annotated genes and result tables for statistical analysis were produced on the base of absolute mapping counts and normalized mapping counts based on transcripts per million (TPM). Key metrics of the final dataset can be reviewed in Table S2 and details on the bioinformatics pipeline, including in-house Shell- and Python scripts are deposited on GitLab (https://gitlab.com/rcmueller/dok-metagenome).

2.5. Soil quality indicators

To link functional gene structure with soil quality, plot specific data on soil quality indicators were retrieved from Krause et al. (2022) and included in the statistical analysis. The samples for this study originate from the first sampling campaign (T1) and were subjected to biogeochemical analysis. In brief, microbial biomass carbon and nitrogen was determined via chloroform fumigation extraction, and basal soil respiration was quantified in a sealed incubation system including acid traps and subsequent titration. Labile carbon (poxC) was determined fluorometrically after oxidation with permanganate. Soil organic carbon and

nitrogen contents were determined via dry combustion and soil total phosphorous contents were quantified with the ignition method. Soil carbon, nitrogen and phosphorous stocks were calculated using plot-specific soil masses of the 0–20 cm layer, based on bulk densities assessment with steel cylinders (n = 5).

2.6. Statistical analysis

Statistical analyses were conducted in R v4.2.0.1 and RStudio v2023.03.0 + 386, and plots were created using the ggplot2 package v.3.4.0 (Wickham Hadley, 2016) unless indicated otherwise. The PHYLOSEQ package v.1.42.0 (McMurdie and Holmes, 2013) was used to combine TPM-normalized count data with hierarchical levels of each classification system and experimental metadata. Before multivariate analysis, genes occurring in less than 10 % of the samples were removed from the dataset to reduce data sparsity (Cao et al., 2021). Between 90 % (EC) and 99 % (PCycDB) of genes were kept for further statistical analysis (Table S3). Effects of cropping systems, experimental plot and sampling time on soil metabolic potential were assessed via permutational multivariate analysis of variance (PERMANOVA) (Anderson, 2001) based on Bray-Curtis dissimilarities using the ADONIS 2 function of the VEGAN package v.2.6.4 (Oksanen et al., 2012) with 10^4 permutations restricted to field blocks. To visualize the impact of experimental factors on soil metabolic potential, a canonical analysis of principal coordinates (Anderson and Willis, 2003) was performed using the CAPdiscrim function implemented in the BiodiveristyR package v.2.15.1 (Kindt and Coe, 2005) constraining for cropping system as the most decisive factor of the PERMANOVA analysis. Biplots with soil quality indicators correlating with the projections of the ordination were calculated using the ENVFIT function of the VEGAN package (Oksanen et al., 2012)and displayed when $R^2 > 0.5$ and $p < 0.001$. *P*-values were corrected for multiple testing via Benjamini-Hochberg.

Indicator genes associated with one or multiple cropping systems were identified using a correlation-based indicator species analysis based on TPM normalized data, independently of time point of sampling. Site-group combinations, as implemented in the MULTIPATT function of the INDICSPECIES package v.1.7.12 (De Cáceres and Legendre, 2009), were enabled with 10^4 permutations. Adjustments for multiple testing were performed using false discovery rate correction with Q-VALUES (Storey and Tibshirani, 2003) implemented in the R package QVALUE v.2.30.0 (Storey and Bass, 2023). For visualization of the cropping system – gene relationships, a bipartite network was generated using Cytoscape v.3.9.1 (Shannon et al., 2003) with genes as target nodes, cropping systems as source nodes, and association strengths (correlation-based indicator value) as connecting edges (Hartmann et al., 2015). An edge-weighted (indicator value) spring-embedded algorithm (Allegro Fruchterman–Reingold layout) was used to visualize treatment-gene associations with q-values <0.001 for all classification systems and additionally, $r^2 > 0.6$ for SEED, PCycDB and CAZy, and $r^2 > 0.7$ for EC.

To quantify the relative change in gene abundances, TPM-normalized data was aggregated for each level in each classification system, independently of time point of sampling. Subsequently, a univariate PERMANOVA was conducted to test for significant effects of cropping system based on Euclidean distance matrices followed by q-value correction. Aggregated read counts were z-transformed using the scale function of base R and treatment-specific means, and standard errors for the highest levels of each classification category were visualized along sums of read counts and means for organic (BIODYN, BIOORG) and conventional (CONFYM, CONMIN) systems.

3. Results

3.1. Soil carbon, nitrogen and phosphorus stocks

The patterns observed in soil carbon and nitrogen stocks were similar, with the highest stocks occurring in the BIODYN treatment, followed by the BIOORG and CONFYM treatments. The CONMIN treatment exhibited significantly diminished carbon and nitrogen stocks in comparison to BIODYN and BIOORG. However, the stocks observed in CONFYM and BIOORG did not show a statistically significant difference. The lowest carbon and nitrogen stocks were identified in the unfertilized NOFERT treatment. A similar trend was observed for phosphorus stocks in CONMIN, which exhibited significantly lower levels compared to BIODYN and CONFYM. Nevertheless, the stocks observed in all fertilized systems did not differ significantly from one another. (Fig. 1).

3.2. Effects of cropping systems on functional gene composition

Bioinformatic analyses resulted in 862, 866, 139 (EC) and 698, 337, 731 (SEED) mapped reads to genes annotated in universal ontologies without any bias of cropping systems (Table S4). Cropping systems consistently altered the composition of functional genes with nuanced differences between ontologies, explaining 43–50% of the observed variance (Table 2). Since no interaction between cropping systems and temporal variability in the spring season was observed, indicative genes and gene classes were assessed independently of time point of sampling (Table 2). For universal ontologies, the compositional differences were mainly driven by the input of farmyard manure with NOFERT and CONMIN being most dissimilar from BIODYN, BIOORG, and CONFYM (Fig. 2). Other system-specific management practices, which essentially distinguish organic from conventional systems, had a smaller effect on functional gene composition, but still distinguished systems receiving mineral fertilizers and pesticides (CONMIN, CONFYM) from those without such inputs (BIODYN, BIOORG, NOFERT). (Fig. 2). The data also showed a compositional gradient within farmyard manure-based systems, mirroring the intensification of manure pre-processing, ranging from stacked manure in CONFYM to rotten manure in BIOORG and composted manure in BIODYN (Fig. 2, Table 1). Ultimately, each cropping system harboured a compositionally unique metagenome (Table S5).

Apart from the cropping systems, functional gene composition was also affected by temporal and spatial variability (Table 2). Temporal variability across the spring vegetation phase explained only 2–4% of the observed variance, indicating minor short-term effects on functional gene composition despite system-specific management practices like fertilization and mechanical weeding in the organic systems. Plot-specific soil quality indicators, such as labile carbon contents (permanganate oxidizable organic carbon - poxC), microbial biomass carbon (C_{mic}), nitrogen (N_{mic}), system-specific nitrogen fertilizer and plant protection inputs highly correlated with functional gene composition across all ontologies (Fig. 2 and Tables S6 and S7).

3.3. Cropping system-sensitive genes and gene classes

The identification of functional traits strongly depends on systematics and hierarchies of gene annotation and clustering to functional gene classes. The EC ontology follows a mechanistic annotation approach with 7 gene classes at the highest hierarchical level, corresponding to essential enzymatic reactions (Bairoch, 2000). *Transferases* were the most abundant gene class and most abundant in the unfertilized system NOFERT (Fig. 3c). *Lyases* and *Oxidoreductases* were enriched in BIOORG and BIODYN, while *Ligases*, *Isomerases*, *Translocases*, and *Hydrolases*, were enriched in the conventional systems CONMIN and CONFYM (Fig. 3b). Bipartite gene-association networks show annotated genes that were enriched in one or more cropping systems at the lowest hierarchical level and facilitate the visualization of shared enrichment of genes between cropping systems. For EC, BIODYN and BIOORG, as well as CONMIN and NOFERT, shared a diverse set of indicative genes, while CONFYM was placed in between and shared genes with both clusters (Fig. 4a, supplement data S1).

The SEED ontology is based on expert-curated subsystems with 13 gene classes on the highest level (Overbeek et al., 2014). Most reads

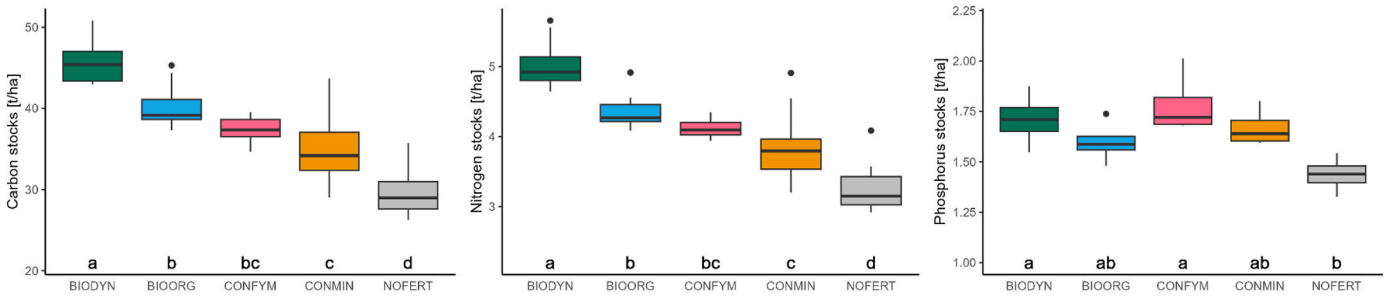

Fig. 1. Mean topsoil (0–20 cm) carbon, nitrogen and phosphorus stocks in spring 2019. Carbon and nitrogen stocks are based on measurements in all subplots (n = 12), while phosphorus stocks are based on measurements in subplot C (n = 4). Posthoc tukey letter indicate significant difference at $p < 0.05$.

Table 2
Effects of cropping systems, experimental plot and temporal variability on functional gene composition as assessed by universal (EC and SEED) and specific (CAZy, NCycDB and PCycDB) ontologies. Effects of main factors and their interactions were assessed by multivariate permutational analysis of variance (PERMANOVA) based on Bray-Curtis dissimilarities retrieved from of TPM-normalized gene counts. Values indicate the explained variance (R^2), the F-ratio (F), and the level of significance (P). *P*-values marked in bold show significant effects at $p < 0.05$

		EC			SEED			CAZy			NCycDB			PCycDB		
	Df	R^2	F	P	R^2	F	P	R^2	F	P	R^2	F	P	R^2	F	P
Cropping System (CS)	4	0.49	32.81	**>0.001**	0.50	40.70	**>0.001**	0.53	39.80	**>0.001**	0.53	48.13	**>0.001**	0.53	36.40	**>0.001**
Experimental plot	15	0.32	5.74	**>0.001**	0.35	7.64	**>0.001**	0.30	6.06	**>0.001**	0.34	8.19	**>0.001**	0.28	5.25	**>0.001**
Temporal variability (TV)	2	0.03	3.61	**0.003**	0.02	4.08	**0.004**	0.03	3.99	**0.002**	0.03	5.03	**0.002**	0.03	4.99	**0.003**
CS * TV	8	0.04	1.39	0.128	0.04	1.56	0.079	0.03	1.25	0.202	0.02	1.08	0.380	0.04	1.55	0.055
Residuals	30	0.11			0.09			0.10			0.08			0.11		

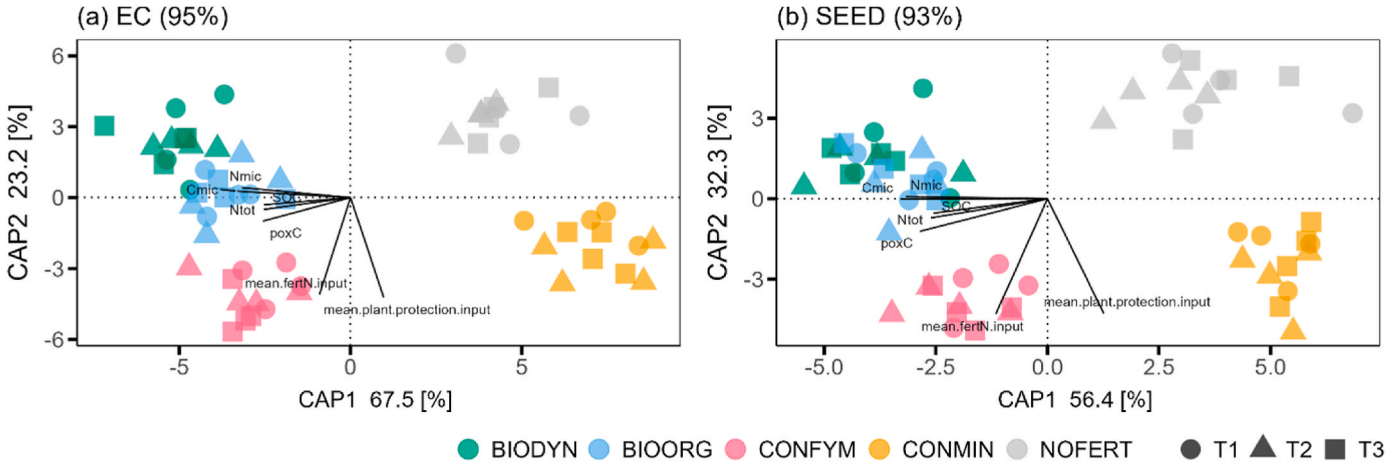

Fig. 2. Canonical Analysis of Principal Coordinates (CAP) based on Bray-Curtis dissimilarities retrieved from TPM-normalized gene counts constrained by the factor cropping system across all three sampling time points. Ordinations are shown for (a) EC and (b) SEED ontologies. The overall reclassification success rate is provided within the plot titles in brackets. Correlating environmental variables are shown for $R^2 > 0.5$ and $p < 0.001$, and include microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}), total soil nitrogen (N_{tot}), soil organic carbon (SOC), permanganate-oxidizable carbon (poxC), system-specific total nitrogen fertilization (mean.fertN.input) and input of active ingredient equivalents for plant protection (mean.plant.protection.input), respectively. Amendment of farmyard manure mainly discerns cropping systems along CAP 1 axis while external input of nitrogen and synthetic plant protection differentiates systems along CAP 2. T1, T2 and T3 refer to soil sampling on February 26th, April 8th and April 15th, 2019, respectively.

were recovered for the *Metabolism* gene class, followed by *Energy* and *Protein processing* (Fig. 3f). Differential abundance of the second hierarchical level of these gene classes are shown in Fig. S2. Genes in the classes *RNA processing*, *Cellular processes* and *Protein processing* were enriched in conventional systems, while the gene classes *Metabolism*, *DNA Processing* and *Miscellaneous* were more abundant in organic systems (Fig. 3e). Within the *Metabolism* gene class, a higher abundance of genes involved in iron, phosphorus, nitrogen and carbon cycling were found in the organic systems. Gene classes associated with nucleotide and amino acid metabolism followed a similar pattern (Fig. S2e). Indicative genes classified within the *Protein processing* subsystem showed a strong association with CONMIN and CONFYM (Fig. 4b) and CONMIN and CONFYM were enriched in functional genes associated with *Protein Synthesis* (Fig. S2h). Functional gene abundance of the *Energy* gene class showed no difference between organic and conventional systems (Fig. 3e), but was especially low abundant in NOFERT (Fig. 3d). On the second hierarchical level of the *Energy* gene class, NOFERT, CONMIN, and to a lesser extent CONFYM, showed enriched genes associated with *Respiration*, while organically fertilized systems, especially BIOORG and BIODYN were enriched in genes associated with *Energy and Precursor Metabolite Generation* (Fig. S2b). Indicative genes of the *Energy* gene class, were mainly linked with aerobic citric acid cycling

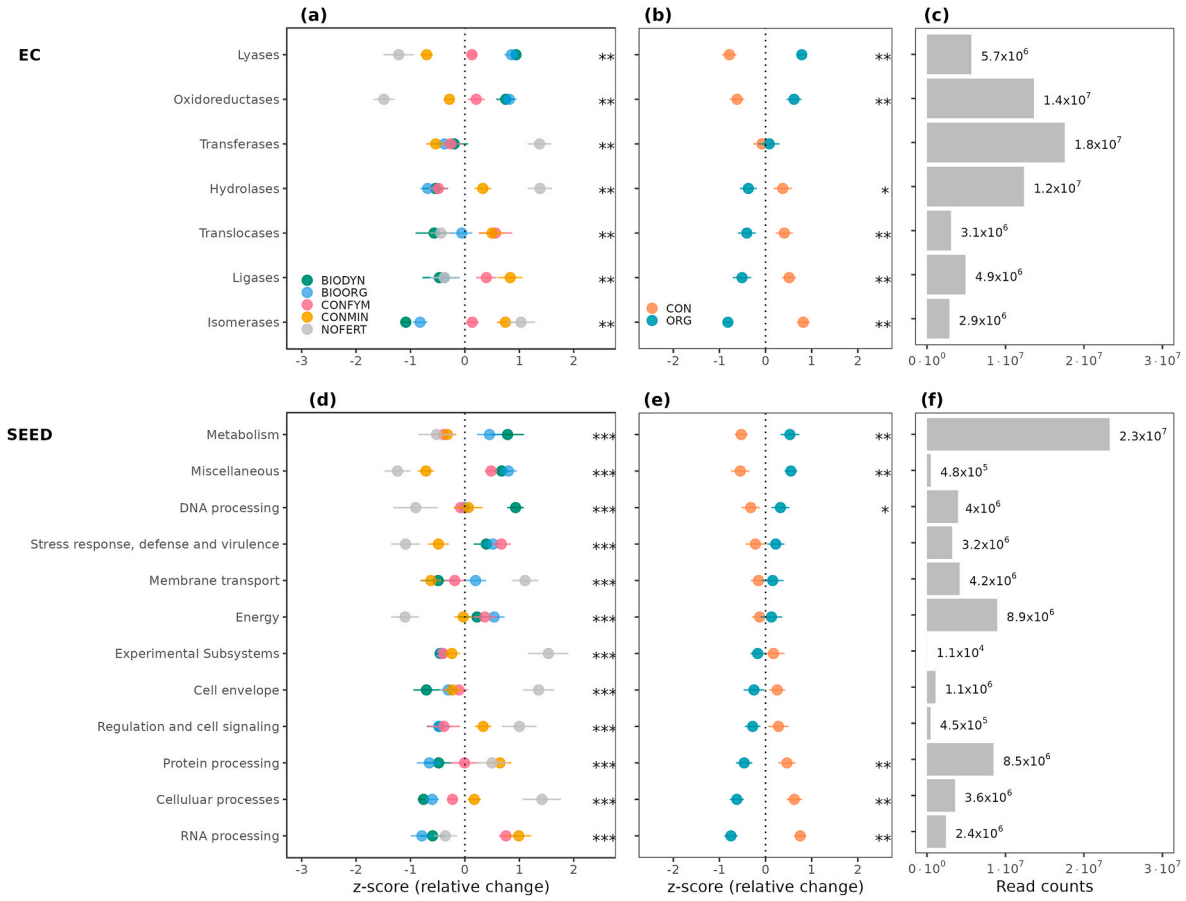

Fig. 3. Relative change in gene class abundance at the highest classification level for EC (a–c) and SEED (*d*–f) classifications based on z-transformed TPM data, independently of time point of sampling. The effect of cropping systems is shown in panels (a) and (d) while the effect of organic (ORG: BIODYN + BIOORG) *versus* conventional (CON: CONFYM + CONMIN) management is shown in panels (b) and (e). The mean and standard error is plotted along with the significance of effects quantified by PERMANOVA and marked as *** for $p < 0.001$, ** for $p < 0.01$ and * for $p < 0.05$. The distribution of read counts per gene class is shown in panels (c) and (f).

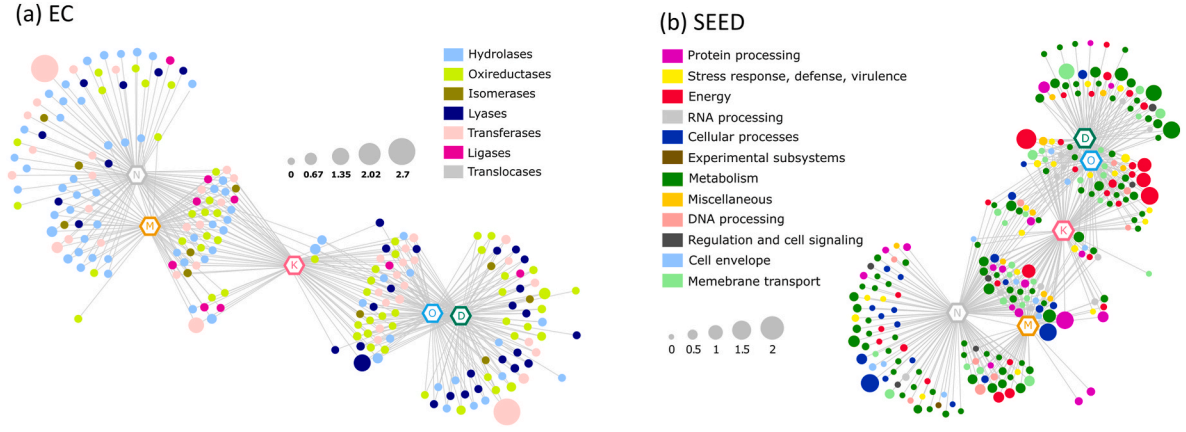

Fig. 4. Bipartite association network of genes indicative for specific cropping systems or system combinations for (a) EC and (b) SEED classification systems, independently of time point of sampling. Diamond-shaped nodes represent cropping systems, circle-shaped nodes represent associated genes and edges show significant positive associations (q-values >0.001, and $R^2 > 0.7$ and $R^2 > 0.6$, for EC and SEED, respectively) between the cropping systems and genes as derived from indicator species analysis. An edge-weighted spring-embedded algorithm was applied to construct the networks with edges weighted according to the association strength. Colors show gene classes at the highest level of hierarchical clustering, while node sizes depict the relative abundance of functional genes. N= NOFERT, D = BIODYN, O = BIOORG, K = CONFYM, M = CONMIN. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

and associated with systems that received farmyard manure, namely CONFYM, BIOORG, and BIODYN (Fig. 4b–supplement data S2). Shared indicative genes from the SEED ontology cluster BIOORG and BIODYN, as well as NOFERT and CONMIN, while CONFYM ranged in between (Fig. 4b).

3.4. Impact of cropping systems on nutrient cycling genes

Bioinformatic analyses yielded 336, 154, 893 (CAZy), 28, 998, 516 (NCycDB), and 114, 994, 738 (PCycDB) mapped reads to genes annotated to nutrient-specific ontologies without any bias of cropping systems (Table S4). All cropping systems showed significantly different compositions of carbon, nitrogen and phosphorus cycling genes, with slight differences between the ontologies (Fig. 5, Table S5). Amendment of farmyard manure was the main factor differentiating NOFERT and CONMIN from BIODYN, BIOORG and CONFYM, especially for CAZy (Fig. 5a).

CAZy distinguishes between six families of carbon cycling enzymes, out of which *Glycoside Hydrolases* and *Glycosyl Transferases* showed the highest read counts and were also significantly enhanced in conventional systems, CONFYM and CONMIN, as well as in the unfertilized control NOFERT (Fig. 6b). Genes associated with *Carbohydrate Binding Modules* and *Polysaccharide Lyases* were principally enriched in the organic systems BIODYN and BIOORG, but also NOFERT showed higher abundances of *Polysaccharide Lyases* compared to conventional systems (Fig. 6a and b). Genes associated with *Auxiliary Activities* were similarly enriched in all farmyard manure-receiving systems and showed the lowest abundances in NOFERT (Fig. 6a). Network analysis of indicative CAZy genes showed a close clustering of BIODYN and BIOORG, and the highest number of genes associated with only one cropping system in NOFERT (Fig. 7a). Interestingly, a cluster of indicative genes annotated as *Carbohydrate Binding Modules* was shared among the organic systems BIODYN and BIOORG, and the unfertilized system NOFERT, which might serve as an indicator for the absence of conventional management with mineral fertilizers and pesticides, next to enriched levels of *Polysaccharide Lyases*.

NCycDB differentiates between eight distinct nitrogen cycling processes, with the majority of read counts associated with the broad category of *Organic degradation and Synthesis*, followed by *Denitrification* and *Assimilatory nitrate reduction* (Fig. 6f). Organic cropping enriched genes for *Denitrification*, *Annamox*, and *Dissimilatory* and *Assimilatory nitrate reduction*, while genes involved in *Nitrogen fixation* and *Organic degradation and Synthesis* were enriched in the conventional systems (Fig. 6e). Although indicative genes for *Nitrification* showed that bacterial *amoA* genes were more strongly associated with CONMIN and CONFYM, and archaeal *amoA* genes were more strongly linked to BIODYN, BIOORG and CONFYM (Fig. 7b), the overall abundances of nitrifying genes were low and did not differ between organic and conventional cropping systems (Fig. 6e).

PCycDB distinguishes between 12 gene classes, out of which the *Two component system* and the *purine metabolism* were the most abundant followed by *Transporters, Pyrimidine metabolism* and *pentose phosphate pathway* (Fig. 6i). Distinct functional gene structure between organic and conventional systems in phosphorus cycling were driven by enriched gene classes associated with *Purine* and *Pyridine metabolism* and *Oxidative phosphorylation* in the conventional systems, and especially CONMIN. Genes associated with the *Pentose phosphate pathway*, *organic phosphoester hydrolysis* and the *Two-component systems* were more abundant in the organic systems BIODYN and BIOORG compared to the conventional systems CONFYM and CONMIN. The *Two-component systems* was especially enriched in NOFERT (Fig. 6g), *while Pyruvate metabolism* and *Transporters* were depleted in this system. The functional gene *phoRPB,* which is associated with the *Two component system* and indicates orthophosphate limitation, was indicative of NOFERT (Fig. 7c). Yet, the highly abundant *phoB* was also associated to the organic systems, as well as the functional gene *pstD*, which is linked to orthophosphate uptake and thus counteracts phosphor limitation. Indicative genes of the PCycDB, such as nucleic acid binding *pur* genes, suggest a higher affinity to purine metabolism in conventional systems. In contrast, a stronger affinity to *organic phosphoester hydrolysis* in organic systems was driven by enriched *phoADX* genes, which encode for alkaline phosphatases.

4. Discussion

4.1. Cropping systems alter soil metabolic potential

Agriculturally managed soils provide key services for food production and regulation of nutrient cycles (Adhikari and Hartemink, 2016). Consequently, the appropriate management of soil and the advancement of sustainable cropping systems that preserve soil functionality represents a key challenge for the agricultural sector. This study demonstrates that the soil metabolic potential and thus soils capacity to provide key services is influenced by organic and conventional cropping systems. While distinct bacterial and fungal community composition (Hartmann et al., 2015) and microbial phenotypes (Esperschütz et al., 2007) have been previously described in the DOK experiment, here we focus on the holistic assessment of functional genetic resources to narrow the knowledge gap between microbial diversity and function, and to identify enriched genes in response to long-term organic and conventional cropping.

The overall pattern in soil metabolic potential, as described by the functional annotation to EC and SEED, was found to be clearly distinguishable for each cropping system. This was particularly evident in the

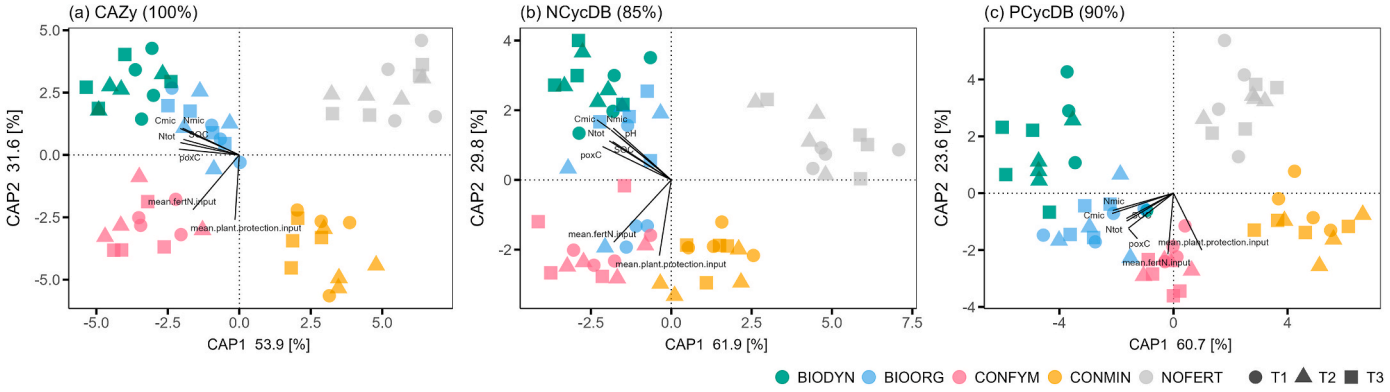

Fig. 5. Canonical Analysis of Principal Coordinates (CAP) based on Bray-Curtis dissimilarities retrieved from TPM-normalized gene counts constrained by the factor cropping system across all three sampling time points. Ordinations are shown for (a) CAZy, (b) NCycDB and (c) PCycDB ontologies. The overall reclassification success rate is provided within the plot titles in brackets. Correlating environmental variables are shown for $R^2 > 0.5$ and $p < 0.001$, and include microbial biomass carbon (C_{mic}) and nitrogen (N_{mic}), total soil nitrogen (N_{tot}), soil organic carbon (SOC), permanganate-oxidizable carbon (poxC), system-specific total nitrogen fertilization (mean.fertN.input) and input of active ingredient equivalents for plant protection (mean.plant.protection.input), respectively. T1, T2 and T3 refer to soil sampling on February 26th, April 8th and April 15th, 2019, respectively.

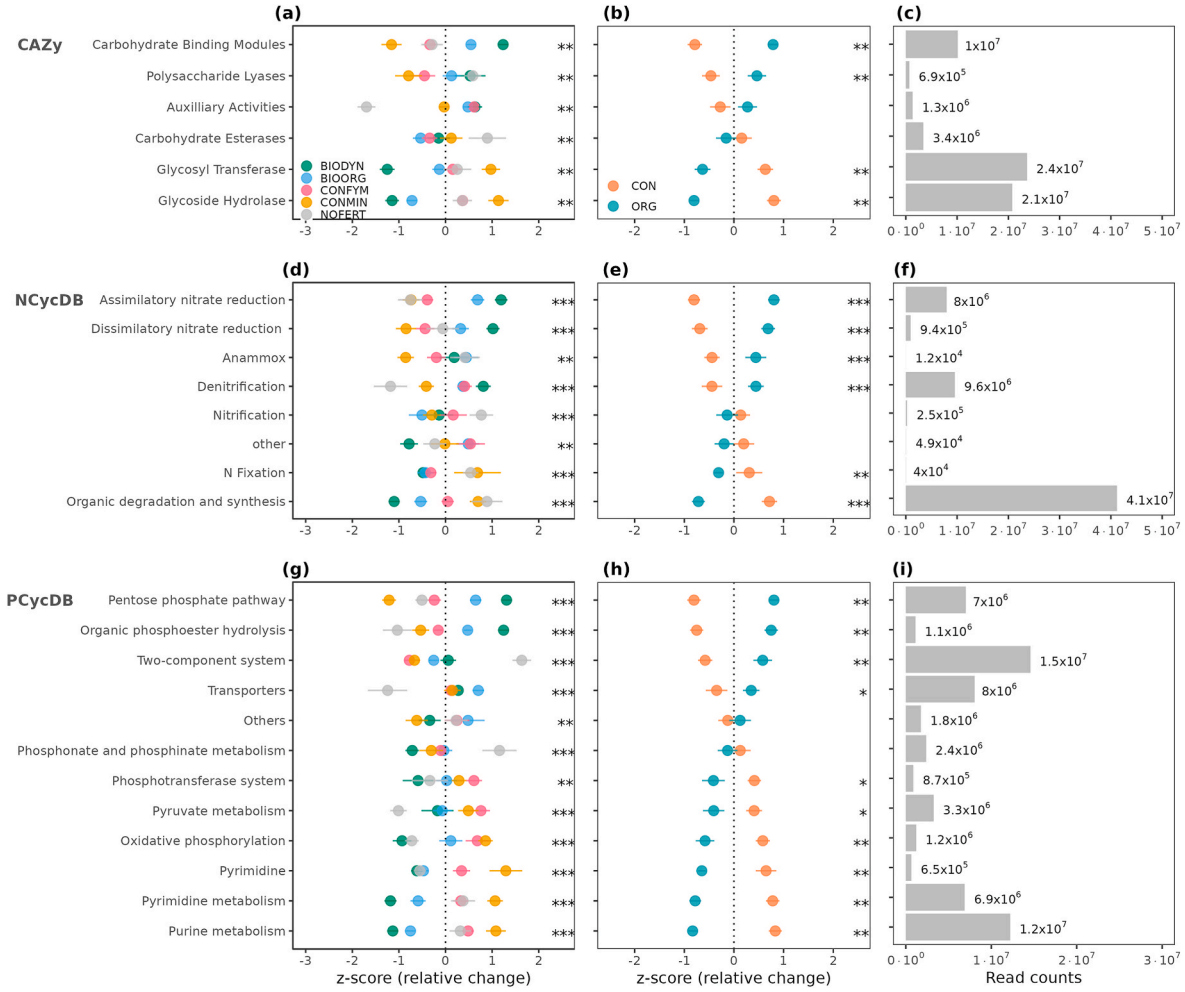

Fig. 6. Relative change in gene class abundance at the highest classification level for CAZy (a–c). NCycDB (*d*–f) and PCycDB (g–i) classifications based on z-transformed TPM data, independently of time point of sampling. The effect of cropping systems is shown in panels (a), (d) and (g) while the effect of organic (ORG: BIODYN + BIOORG) *versus* conventional (CON: CONFYM + CONMIN) management is shown in panels (b), (e) and (h). The mean and standard error is plotted along with the significance of effects quantified by PERMANOVA and marked as *** for $p < 0.001$, ** for $p < 0.01$ and * for $p < 0.05$. The distribution of read counts per category is shown in panels (c), (f) and (i).

case of farmyard manure receiving systems BIODYN, BIOORG and CONFYM, which exhibited notable differences from CONMIN and NOFERT. However, the management practices that distinguish organic from conventional systems, such as inputs of mineral nitrogen fertilizer and synthetic plant protection products, were also identified as important drivers for the observed differences in the soil metabolic potential. The apparent gradient in soil metabolic potential within the farmyard manure receiving systems, from BIODYN to BIOORG to CONFYM, probably reflects system-specific manure processing and storage. The quality of manure has been demonstrated to modulate soil carbon dynamics, especially of the particulate organic matter fractions, which are closely linked to soil microbial metabolism (Mayer et al., 2022). Furthermore, strong correlations have been observed between the soil metabolic potential and soil quality indicators related to carbon and nitrogen, such as microbial biomass carbon and nitrogen, soil organic carbon, total nitrogen and poxC. These findings substantiate the pivotal role of organic matter mineralization in shaping the metabolic potential of the soil microbiome.

Despite different management interventions such as fertilization during the spring sampling period, the temporal effects on soil metabolic potential remained subordinate and less decisive compared to spatial effects and the long-term impact of cropping systems. This is consistent with a recent study, in which seasonal variability had lesser impact compared to the influence of soil sampling location, as analysed by a metagenome assembled genome approach (Orellana et al., 2018). However, as DNA extraction did not differentiate between living and deceased cells, relic DNA could have contributed to the detected soil metabolic potential across different time points (Carini et al., 2016). Therefore, the observed soil metabolic potential may partly comprise the legacy effect of agricultural history, possibly masking the short-term effect induced by fertilization.

4.2. Cropping systems affect cycling of exogenous nutrients and cellar processes

Gene classification systems offer insights into the functional potential of microbial communities depending on the hierarchical levels of gene classes. While the SEED ontology uses hand-curated subsystems to group functional genes, the EC ontology follows a mechanistic classification approach, meaning that enzymes catalysing the same reaction might originate from different organisms and differ in their genetic code, but will still be assigned to the same EC number (Bairoch, 2000).

The augmented capacity for carbohydrate, nucleotide and amino acid metabolism in organically fertilized systems, as observed within the SEED Metabolism subsystems, lends support to the notion of a metabolic adaptation to exogenous organic matter sources. Furthermore, a higher capacity for iron and phosphate metabolism under organic management was observed (Figs. S2d and e). Since iron is recognized as a redox-

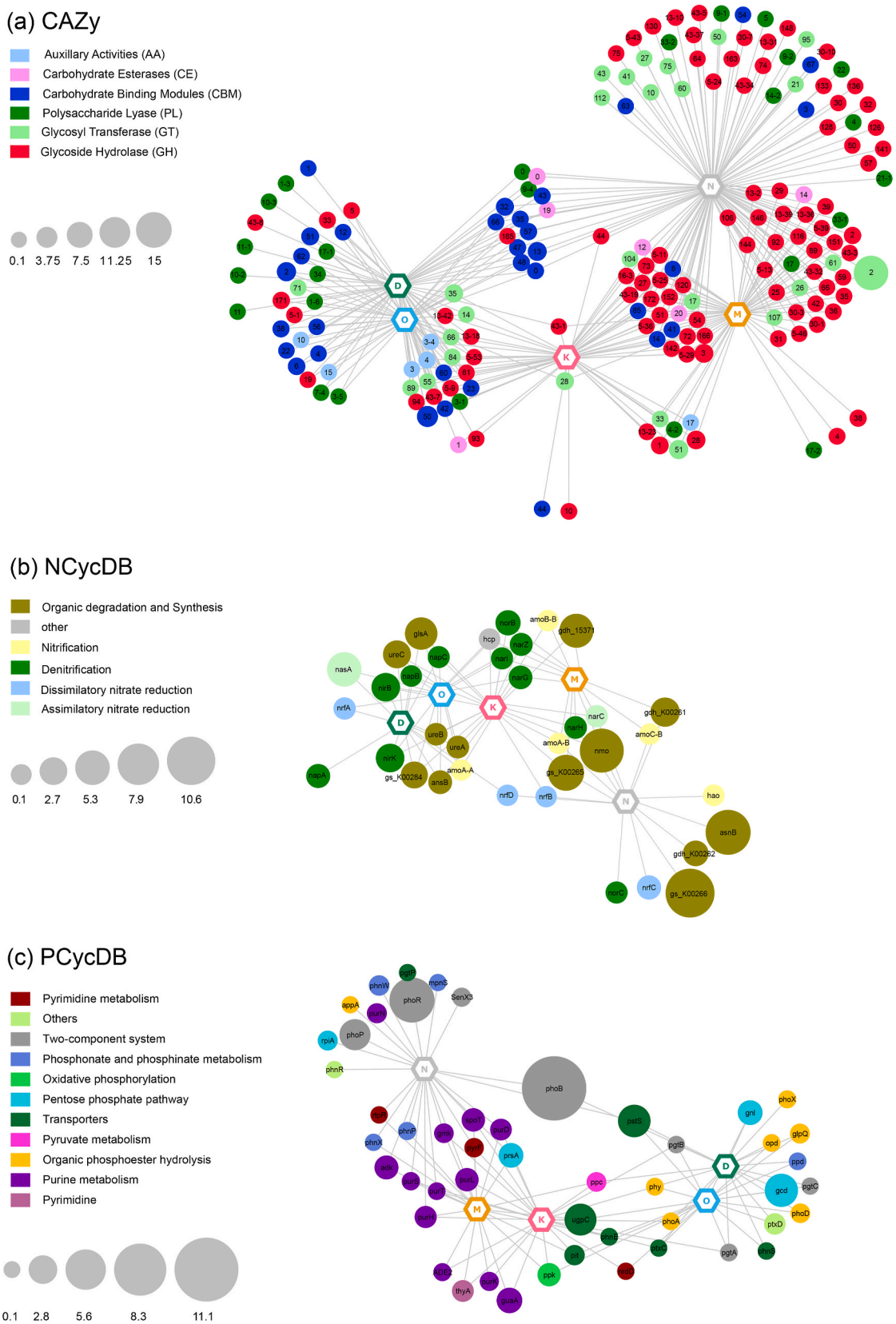

Fig. 7. Bipartite association network of genes indicative for specific cropping systems or system combinations for (a) CAZy, (b) NCycDB and (c) PCycDB classification systems, independently of time point of sampling. Diamond-shaped nodes represent cropping systems, circle-shaped nodes represent associated genes, and edges show significant (q-values >0.001 and $R^2 > 0.6$) positive associations between the cropping systems and genes as derived from indicator species analysis. An edge-weighted spring-embedded algorithm was applied to construct the networks with edges weighted according to the association strength. Colors show gene classes at the first level of hierarchical clustering, while node sizes depict relative abundance of functional genes. Node labels correspond to gene classifications according to CAZy, NCycDB and PCycDB (Tu et al., 2019; Drula et al., 2022; Zeng et al., 2022). N = NOFERT, D = BIODYN, O = BIOORG, K = CONFYM, M = CONMIN.

sensitive transition element that is closely associated with other elemental cycles (Kappler et al., 2021), this observation is consistent with elevated levels of *Oxidoreductases*, as categorized by the EC ontology. Genes encoding *Oxidoreductases* and *Lyases* were found to be more abundant in organic cropping systems and *Lyases* catalyse non-hydrolytic elimination reactions, which often result in the formation of new double bonds or ring structures. The shift from high hydrolase abundance in CONMIN and NOFERT towards the enrichment of *Lyases* in the systems receiving farmyard manure (CONFYM, BIOORG and BIODYN) implies a fundamental alteration in organic matter decomposition mechanism from hydrolytic towards non-hydrolytic carbohydrate processing upon exogenous organic matter input. Interestingly, genes associated with *Stress response, defense and virulence* were also enriched in systems receiving farmyard manure, which suggests that organic inputs raise stress on the indigenous soil community, possibly through enhanced competition of introduced microbes or substances that induce additional pressure on cell functioning. However, this hypothesis needs further clarification, e.g., by studying microbial compositions in organic fertilizers and their fate upon soil application.

In contrary, predictive gene classes for the minerally fertilized systems point towards accelerated cell metabolism and transcriptional activity with enriched capacity for *Cellular processes*, *RNA processing* and *Protein processing,* according to the SEED ontology (Fig. 3d and e). For the latter, indicative functional genes were mainly assigned to *Protein synthesis* on the second annotation level, which points towards a higher capacity for *de-novo* protein synthesis in conventional systems likely due to higher nitrogen inputs. Higher shares of *Isomerases* and *Translocases* in the conventional systems indicate reinforced isomeric modification and active transport of molecules through cell membranes. Together with the increased abundance of *Ligases*, which catalyse the energy-demanding formation of covalent bonds, indicative enzyme classes for conventional systems associate with molecule uptake and raised cellular energy demands through ligase activity. This is well in line with the observation that the ratio of copiotrophic to oligotrophic bacteria declined from CONFYM to BIOORG and BIODYN, based on a previous taxonomic identification (Lori et al., 2023). Yet, significantly lower microbial biomass carbon in conventional compared to organic systems of the DOK experiment (Krause et al., 2022) questions the direct translation of protein synthesizing capacity into microbial growth.

4.3. Organic fertilization induces shifts in carbohydrate cycling capacities

The CAZy ontology facilitates identification of gene classes that are responsive to cropping systems and offers a mechanistic insight in changes of carbon metabolism to understand distinct soil organic carbon stocks after 42 years of management. *Carbohydrate Binding Modules* are involved in the non-catalytic binding of complex carbohydrates such as starch, pectin and cellulose, and support catalytic modules such as *Polysaccharide Lyases*, *Glycoside Hydrolases* or *Auxiliary Activities* (Guillén et al., 2010). *Polysaccharide Lyases* describe a rather narrow functional group of enzymes with high dependency on *Carbohydrate Binding Modules* (Garron and Cygler, 2014). Indeed, both gene classes showed enriched abundances in organic systems, indicating an enhanced capacity for processing of rather complex organic carbon compounds. Both gene classes well reflect differences in soil organic carbon stocks between the fertilized systems, but high levels of *Polysaccharide Lyases* in NOFERT also suggests nutrient limited conditions for favor high abundances of *Polysaccharide Lyases* (Fig. 6a and b). However, abundances of *Carbohydrate Binding Modules* best reflected the observed trends in soil organic carbon between cropping systems with highest abundances in BIODYN, followed by BIOORG and CONFYM. Although not being catalytically active, *Carbohydrate Binding Modules* have the ability to target their parent proteins to specific carbohydrate substrates and thus might serve as indicator for the quality of long-term organic matter inputs. Also, Enzymes encoded by *Auxiliary Activities* contain redox-active enzymes that are related to lignocellulose conversion, and are therefore crucial for the degradation of complex organic matter compounds (Levasseur et al., 2013). Yet, genes associated with *Auxiliary Activities* were equally enriched in all systems receiving organic inputs, namely CONFYM, BIOORG and BIODYN, and could thus serve as an indicator for the legacy of organic fertilization.

Furthermore, fundamentally distinct catalytic capacities were observed between organic and conventional systems. Non -hydrolytic carbohydrate processing capacity via *Polysaccharide Lyases* was enriched in organically fertilized systems, in contrast to higher capacity for hydrolytic cleavage of carbohydrates via *Glycoside Hydrolases* in conventional system. The functionally diverse gene class of *Glycoside Hydrolases* utilizes water to catalyse the hydrolysis of glycosidic bonds, forming mostly hemiacetal sugars and aglycons (Vocadlo and Davies, 2008). Together with *Glycosyl Transferases*, *Glycoside Hydrolases* form the machinery for the breakage and synthesis of glycosidic bonds, which also includes binding of nitrogen-containing amino groups. The relative enrichment of both gene classes in conventional systems pinpoint a higher capacity to process low-complexity carbohydrates and amino bonds.

The functional differences observed in carbohydrate-active enzymes between organic and conventional cropping systems demonstrate the presence of distinct carbon cycling mechanisms. These mechanisms appear to favor glycoside cycling in conventional systems and an enhanced capacity to bind and degrade complex lignocellulose compounds in the organically fertilized systems, particularly in the BIOORG and BIODYN systems.

4.4. Organic cropping system enhance capacity for nitrate cycling

Nitrogen fertilization is a major driver of yields and across the five main crops winter wheat, grassclover, maize soybean and potato, yields in organic systems were 15% lower than in conventional systems (Knapp et al., 2023). Due to the absence of mineral nitrogen fertilization, soil nitrogen cycling is especially crucial for organic systems. Despite lower inputs organic systems maintained or raised soil nitrogen stocks, which suggest distinct nitrogen cycling capacities between cropping systems. The high share of genes that associated with the broad category of *Organic degradation and Synthesis* underpin the importance of the soil organic nitrogen pool and the critical role of soil microbes in the assimilation and mineralization of organic nitrogen compounds. The abundance of genes related to *Organic degradation and Synthesis* is inversely proportional to long-term trends in soil nitrogen stocks (Oberson et al., 2024). Indicative genes for the conventional systems of this gene class, such as gs_K00265 (*glt*) and gdh_15,371 (Fig. 7b), encode for glutamate synthase and glutamate dehydrogenase (Tu et al., 2019) and point towards enhanced mineral nitrogen assimilation, mainly regulated through enhanced ammonium availability. This may be directly related to the continuous mineral fertilization with calcium ammonium sulphate, which stimulates the microbial capacity to take up ammonia for biosynthesis. In addition, the *nmo* gene encode for nitronate monooxygenase, and showed which is crucial for detoxifying nitroalkanes (Torres-Guzman et al., 2021) was also observed as an indicative gene for herbicide resistance in plants (Gaines et al., 2014). While this suggests that herbicide inputs in conventional systems induced the enrichment of *nmo* genes, NOFERT also showed a high abundance of *nmo* despite never receiving synthetic plant protection inputs (Fig. 7b). Although this gene contributes to the observed differences in nitrogen cycling capacity between organic and conventional systems, its possible functions remain manifold and to be uncovered (Torres-Guzman et al., 2021).

Genes associated to the highly abundant gene classes *Assimilatory nitrate reduction* were enriched in organic systems, which indicate a greater potential for retaining nitrogen within the soil system. Especially the high abundance of the *nasA* gene (Fig. 7b), which encodes for a nitrate transporter (Ogawa et al., 1995), suggests an increased genetic capacity for selective nitrate acquisition in organic systems, which might

be driven by the need to cope with nitrogen-limiting conditions, and possibly contributes to the mitigation of nitrate leaching.

Abundance of nitrifying genes did not differentiate between organic and conventional cropping systems, but indicative genes coding for nitrification suggest taxonomic niche separation for archaeal and bacterial nitrifiers. This is in line with in-depth studies on ammonia oxidation kinetics showing that bacterial nitrifiers benefit from mineral nitrogen sources, while archaeal nitrifiers thrive on organic nitrogen sources (Martens-Habbena et al., 2009). In addition, cropping systems were recently identified as an important driver for a distinct community structure of ammonia oxidisers and their response to drought in the DOK experiment (Bintarti et al., 2025). The highest share of genes encoding for *Nitrogen fixation* was observed in the purely mineral-fertilized CONMIN and the unfertilized NOFERT systems (Fig. 6d), However, it needs to be noted that, symbiotic nitrogen fixation during grassclover and soy cropping was lower in CONMIN (99 kg N ha^{-1} yr^{-1}) compared to CONFYM (117 kg N ha^{-1} yr^{-1}), BIOORG (119 kg N ha^{-1} yr^{-1}) and BIODYN (122 kg N ha^{-1} yr^{-1}) (Oberson et al., 2024). Since nitrogen fixation of free living nitrogen fixers is estimated not to exceed 20 kg N ha^{-1} yr^{-1} (Cleveland et al., 2022), it seems evident that the proliferation of nitrogen-fixing microbes is enhanced during symbiotic living stages, which cannot be addressed comprehensively with the analysis of bulk soil within this study.

Genes associated with *Denitrification* were enriched in the manure-receiving systems BIOORG, BIODYN and CONFYM, which corresponds well to the need for carbon sources for this heterotrophic process (Fig. 6e and f). Denitrification drives the loss of gaseous nitrogen species from the soil system, and especially high abundances of *nirK*, *nirB* and *norB* (Fig. 7b) suggest enhanced formation of the greenhouse gas nitrous oxide by facilitating nitrite and nitric oxide reduction. Although these genes were highly abundant in all organically fertilized systems, nitrous oxide emissions in a grassclover-maize-green manure cropping sequence were lowest in BIODYN, and the highest emissions were found in CONFYM (Skinner et al., 2019). While highest nitrogen inputs in CONFYM can be considered as the most important driver for this observation, also cropping system induced differences in soil pH were shown to affect nitrous oxide production and reduction rates (Krause et al., 2017). This highlights that the soil genetic potential not necessarily reflects actual gene expression or process rates and the metabolic potential of the soil ultimately needs to be harnessed by agricultural management.

4.5. Phosphorus cycling as affected by cropping systems

In organic systems, the provision of phosphorus is particularly challenging as the high reliance on biological nitrogen fixation in organic farms often cannot be stoichiometrically balanced with phosphorus rich organic fertilizers and thus is often associated with phosphorus export (Reimer et al., 2020). In conventional systems, plant phosphorus requirements are met directly by mineral inputs, which favours phosphorus cycling processes associated with *Pyrimidine* and *Purine metabolism*. Both classes of genes are involved in the formation of DNA and RNA, and their metabolites provide cells with energy and support their survival and proliferation. This observation is well in line with the increased capacity for RNA and protein processing in the conventional system as identified by the SEED ontology (Fig. 3d and e), and further underscores a trend towards increased cellular activity resulting from conventional soil management. However, DNA processing and nucleotide processing was enhanced in organic fertilized systems (Figs. S2d and e), which indicates that enhanced pyrimidine and purine metabolism in conventional systems rather aligns with RNA processing but not with DNA metabolism.

While long-term nitrogen requirements in organic cropping systems are secured by nitrogen-fixing legumes, there is a lack of organic fertilizers capable of balancing crop nitrogen and phosphorus requirements. However, the effect of lower phosphorus inputs in organic systems could be partly compensated by increased phosphatase activity, which cleaves organically bound phosphorus and makes it potentially available for plant uptake. This hypothesis is supported by higher abundances of functional genes related to the *Pentose phosphate pathway* and *Organic phosphoester hydrolysis* in the organic system (Fig. 6g and h). In particular, the alkaline phosphatase encoding gene *phoAXD* indicates a higher potential for phosphorus acquisition from organic sources (Fig. 7c), which is in good agreement with the observed higher activity of alkaline phosphatases in the soils of the DOK experiment (Krause et al., 2022). Also, distinct community structure of arbuscular mycorrhizal fungi between CONMIN and BIODYN, coupled with a higher abundance of in BIODYN, was recently observed in the DOK experiment (Kundel et al., 2020), which further indicates enhanced phosphate acquisition as an adaptive mechanism to phosphorus limited conditions.

Additionally, the functional gene *gcd,* which encodes for quinoprotein glucose dehydrogenase, and was found to be enriched in organic systems (Fig. 7c), has previously been identified as a marker gene for inorganic phosphorus solubilization and bioavailable soil phosphorus (Wu et al., 2022). In contrast, the functional gene *phoB*, which indicates phosphorus limitation (Xu et al., 2012), and the transporter gene *pst*S were associated with organic and unfertilized systems (Fig. 7c). This association can be interpreted as a response to the lower phosphorus inputs in organic systems. Although plant tissue analyses did not indicate any symptoms of plant phosphorus deficiency in the systems of the DOK experiment (Oberson et al., 2007, 2013), these results highlight the necessity of ensuring long-term phosphorus demand in organic systems through the use of phosphorus-enriched recycled fertilizers.

5. Conclusion

The amendment of farmyard manure was the main factor differentiating the soil metabolic potential between cropping systems, followed by nitrogen and plant protection inputs, as well as manure preprocessing. While conventional cropping systems were enriched by genes indicating the adaptation to use inorganic nutrients and improve cellular processes such as molecule transport, modification and synthesis, the metabolic potential of organically managed soils was dominated by genes crucial for degradation of complex organic compounds, nitrate assimilation, and organic phosphorus acquisition. The findings of this study demonstrate that cropping systems exert a significant influence on the soil metabolic potential, particularly in regard to their capacity to cycle carbon, nitrogen and phosphorus and thus drive long-term developments in soil stocks. In order to facilitate the advancement of sustainable cropping systems, it is essential to harness the long-term impact of such systems on soil metabolic potential, with the aim of steering the inherent multifunctionality of soil biological resources for the benefit of environmental health and agricultural production.

CRediT authorship contribution statement

Hans-Martin Krause: Writing – original draft, Visualization, Methodology, Funding acquisition, Conceptualization. **Ralf C. Mueller:** Writing – review & editing, Software, Methodology, Data curation. **Martina Lori:** Writing – review & editing, Visualization, Methodology. **Jochen Mayer:** Writing – review & editing, Conceptualization. **Paul Mäder:** Writing – review & editing, Funding acquisition, Conceptualization. **Martin Hartmann:** Writing – review & editing, Visualization, Methodology, Conceptualization.

Data availability

Rawdata is deposited in NCBI's Sequence Read Archive (PRJNA924160) and the documentation of the bioinformatics pipeline can be retrieved from gitlab. com/rcmueller/dok-metagenome.

Declaration of competing interest

The authors declare no competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgments

This work was supported by the Swiss National Science Foundation (grant number 31003 A_182390). Also, we want to thank the Swiss Federal Office of Agriculture (FOAG) for its continuous support of the DOK long-term experiment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.soilbio.2025.109737.

References

Adhikari, K., Hartemink, A.E., 2016. Linking soils to ecosystem services - a global review. Geoderma 262, 101–111. https://doi.org/10.1016/j.geoderma.2015.08.009.

Agarwala, R., Barrett, T., Beck, J., Benson, D.A., Bollin, C., Bolton, E., Bourexis, D., Brister, J.R., Bryant, S.H., Canese, K., Cavanaugh, M., Charowhas, C., Clark, K., Dondoshansky, I., Feolo, M., Fitzpatrick, L., Funk, K., Geer, L.Y., Gorelenkov, V., Graeff, A., Hlavina, W., Holmes, B., Johnson, M., Kattman, B., Khotomlianski, V., Kimchi, A., Kimelman, M., Kimura, M., Kitts, P., Klimke, W., Kotliarov, A., Krasnov, S., Kuznetsov, A., Landrum, M.J., Landsman, D., Lathrop, S., Lee, J.M., Leubsdorf, C., Lu, Z., Madden, T.L., Marchler-Bauer, A., Malheiro, A., Meric, P., Karsch-Mizrachi, I., Mnev, A., Murphy, T., Orris, R., Ostell, J., O'Sullivan, C., Palanigobu, V., Panchenko, A.R., Phan, L., Pierov, B., Pruitt, K.D., Rodarmer, K., Sayers, E.W., Schneider, V., Schoch, C.L., Schuler, G.D., Sherry, S.T., Siyan, K., Soboleva, A., Soussov, V., Starchenko, G., Tatusova, T.A., Thibaud-Nissen, F., Todorov, K., Trawick, B.W., Vakatov, D., Ward, M., Yaschenko, E., Zasypkin, A., Zbicz, K., 2018. Database resources of the national center for biotechnology information. Nucleic Acids Research 46, D8–D13. https://doi.org/10.1093/nar/gkx1095.

Anderson, M.J., 2001. A new method for non-parametric multivariate analysis of variance. Austral Ecology 32–46.

Anderson, M.J., Willis, T.J., 2003. Canonical analysis of principal coordinates: a useful method of constrained ordination for ecology. Ecology 84, 511–525. https://doi.org/10.1890/0012-658(2003)084[0511:CAOPCA]2.0.CO;2.

Andrews, S., 2012. Fast QC: a quality control tool for high throughput sequence data. github.com/s-andrews/FastQC.

Bairoch, A., 2000. The ENZYME database in 2000. Nucleic Acids Research 28, 304–305. https://doi.org/10.1093/nar/28.1.304.

Banerjee, S., van der Heijden, M.G.A., 2023. Soil microbiomes and one health. Nature Reviews Microbiology 21, 6–20. https://doi.org/10.1038/s41579-022-00779-w.

Bintarti, A.F., Kost, E., Kundel, D., Conz, R.F., Mäder, P., Krause, H.M., Mayer, J., Philippot, L., Hartmann, M., 2025. Cropping system modulates the effect of spring drought on ammonia-oxidizing communities. Soil Biology and Biochemistry 201. https://doi.org/10.1016/j.soilbio.2024.109658.

Buchfink, B., Reuter, K., Drost, H.G., 2021. Sensitive protein alignments at tree-of-life scale using DIAMOND. Nature Methods 18, 366–368. https://doi.org/10.1038/s41592-021-01101-x.

Bünemann, E.K., Bongiorno, G., Bai, Z., Creamer, R.E., De Deyn, G., de Goede, R., Fleskens, L., Geissen, V., Kuyper, T.W., Mäder, P., Pulleman, M., Sukkel, W., van Groenigen, J.W., Brussaard, L., 2018. Soil quality – a critical review. Soil Biology and Biochemistry 120, 105–125. https://doi.org/10.1016/j.soilbio.2018.01.030.

Cao, Q., Sun, X., Rajesh, K., Chalasani, N., Gelow, K., Katz, B., Shah, V.H., Sanyal, A.J., Smirnova, E., 2021. Effects of rare microbiome taxa filtering on statistical analysis. Frontiers in Microbiology 11, 1–15. https://doi.org/10.3389/fmicb.2020.607325.

Carini, P., Marsden, P.J., Leff, J.W., Morgan, E.E., Strickland, M.S., Fierer, N., 2016. Relic DNA is abundant in soil and obscures estimates of soil microbial diversity. Nature Microbiology 2, 16242. https://doi.org/10.1038/nmicrobiol.2016.242.

Chen, S., Zhou, Y., Chen, Y., Gu, J., 2018. Fastp: an ultra-fast all-in-one FASTQ preprocessor. Bioinformatics 34, i884–i890. https://doi.org/10.1093/bioinformatics/bty560.

Cleveland, C.C., Reis, C.R.G., Perakis, S.S., Dynarski, K.A., Batterman, S.A., Crews, T.E., Gei, M., Gundale, M.J., Menge, D.N.L., Peoples, M.B., Reed, S.C., Salmon, V.G., Soper, F.M., Taylor, B.N., Turner, M.G., Wurzburger, N., 2022. Exploring the role of cryptic nitrogen fixers in terrestrial ecosystems: a frontier in nitrogen cycling research. Ecosystems 25, 1653–1669. https://doi.org/10.1007/s10021-022-00804-2.

Danecek, P., Bonfield, J.K., Liddle, J., Marshall, J., Ohan, V., Pollard, M.O., Whitwham, A., Keane, T., McCarthy, S.A., Davies, R.M., 2021. Twelve years of SAMtools and BCFtools. GigaScience 10, 1–4. https://doi.org/10.1093/gigascience/giab008.

De Cáceres, M., Legendre, P., 2009. Associations between species and groups of sites: indices and statistical inference. Ecology 90, 3566–3574. https://doi.org/10.1890/08-1823.1.

de la Cruz, V.Y.V., Tantriani, Cheng, W., Tawaraya, K., 2023. Yield gap between organic and conventional farming systems across climate types and sub-types: a meta-analysis. Agricultural Systems 211, 103732. https://doi.org/10.1016/j.agsy.2023.103732.

Drula, E., Garron, M.L., Dogan, S., Lombard, V., Henrissat, B., Terrapon, N., 2022. The carbohydrate-active enzyme database: functions and literature. Nucleic Acids Research 50, D571–D577. https://doi.org/10.1093/nar/gkab1045.

Edgar, R.C., 2010. Search and clustering orders of magnitude faster than BLAST. Bioinformatics 26, 2460–2461. https://doi.org/10.1093/bioinformatics/btq461.

Esperschütz, J., Gattinger, A., Mäder, P., Schloter, M., Fließbach, A., 2007. Response of soil microbial biomass and community structures to conventional and organic farming systems under identical crop rotations. FEMS Microbiology Ecology 61, 26–37. https://doi.org/10.1111/j.1574-6941.2007.00318.x.

Foley, J.A., DeFries, R., Asner, G.P., Barford, C., Bonan, G., Carpenter, S.R., Chapin, F.S., Coe, M.T., Daily, G.C., Gibbs, H.K., Helkowski, J.H., Holloway, T., Howard, E.A., Kucharik, C.J., Monfreda, C., Patz, J.A., Prentice, I.C., Ramankutty, N., Snyder, P.K., 2005. Global consequences of land use. Science 309, 570–574. https://doi.org/10.1126/science.1111772.

Gaines, T.A., Lorentz, L., Figge, A., Herrmann, J., Maiwald, F., Ott, M.C., Han, H., Busi, R., Yu, Q., Powles, S.B., Beffa, R., 2014. RNA-Seq transcriptome analysis to identify genes involved in metabolism-based diclofop resistance in Lolium rigidum. The Plant Journal 78, 865–876. https://doi.org/10.1111/tpj.12514.

Garron, M.L., Cygler, M., 2014. Uronic polysaccharide degrading enzymes. Current Opinion in Structural Biology 28, 87–95. https://doi.org/10.1016/j.sbi.2014.07.012.

Gough, J., Karplus, K., Hughey, R., Chothia, C., 2001. Assignment of homology to genome sequences using a library of hidden Markov models that represent all proteins of known structure. Journal of Molecular Biology 313, 903–919. https://doi.org/10.1006/jmbi.2001.5080.

Guillén, D., Sánchez, S., Rodríguez-Sanoja, R., 2010. Carbohydrate-binding domains: multiplicity of biological roles. Applied Microbiology and Biotechnology 85, 1241–1249. https://doi.org/10.1007/s00253-009-2331-y.

Hartmann, M., Frey, B., Mayer, J., Mäder, P., Widmer, F., 2015. Distinct soil microbial diversity under long-term organic and conventional farming. ISME Journal 9, 1177–1194. https://doi.org/10.1038/ismej.2014.210.

Hartmann, M., Six, J., 2023. Soil structure and microbiome functions in agroecosystems. Nature Reviews Earth & Environment 4, 4–18. https://doi.org/10.1038/s43017-022-00366-w.

Huang, L., Zhang, H., Wu, P., Entwistle, S., Li, X., Yohe, T., Yi, H., Yang, Z., Yin, Y., 2018. DbCAN-seq: a database of carbohydrate-active enzyme (CAZyme) sequence and annotation. Nucleic Acids Research 46, D516–D521. https://doi.org/10.1093/nar/gkx894.

Huson, D.H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S., Ruscheweyh, H.J., Tappu, R., 2016. MEGAN community edition - interactive exploration and analysis of large-scale microbiome sequencing data. PLoS Computational Biology 12, 1–12. https://doi.org/10.1371/journal.pcbi.1004957.

Hyatt, D., Chen, G.-L., LoCascio, P.F., Land, M.L., Larimer, F.W., Hauser, L.J., 2010. Prodigal: prokaryotic gene recognition and translation initiation site identification. BMC Bioinformatics 11, 119. https://doi.org/10.1186/1471-2105-11-119.

IPBES, 2019. In: Brondizio, E.S., Settele, J., Díaz, S., Ngo, H.T. (Eds.), Global Assessment Report on Biodiversity and Ecosystem Services of the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services. IPBES secretariat, Bonn, Germany, p. 1148. https://doi.org/10.5281/zenodo.3831673.

IPCC, 2023. Climate change 2023: synthesis report. Contribution of working groups I. In: Lee, H., Romero, J. (Eds.), II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team. IPCC, Geneva, Switzerland, pp. 35–115. https://doi.org/10.59327/IPCC/AR6-9789291691647.

Kappler, A., Bryce, C., Mansor, M., Lueder, U., Byrne, J.M., Swanner, E.D., 2021. An evolving view on biogeochemical cycling of iron. Nature Reviews Microbiology 19, 360–374. https://doi.org/10.1038/s41579-020-00502-7.

Kindt, R., Coe, R., 2005. Tree Diversity Analysis A Manual and Software for Common Statistical Methods for Ecological and Biodiversity Studies. World Agroforestry Centre (ICRAF), Nairobi.

Knapp, S., Gunst, L., Mäder, P., Ghiasi, S., Mayer, J., 2023. Organic cropping systems maintain yields but have lower yield levels and yield stability than conventional systems – results from the DOK trial in Switzerland. Field Crops Research 302. https://doi.org/10.1016/j.fcr.2023.109072.

Knapp, S., van der Heijden, M.G.A., 2018. A global meta-analysis of yield stability in organic and conservation agriculture. Nature Communications 9, 1–9. https://doi.org/10.1038/s41467-018-05956-1.

Krause, H.-M., Fliessbach, A., Mayer, J., M, P., 2020. Chapter 2 - implementation and management of the DOK long-term system comparison trial. In: Bhullar, G.S., Riar, A. (Eds.), Long-Term Farming Systems Research. Academic Press, pp. 37–51. https://doi.org/10.1016/B978-0-12-818186-7.00003-5.

Krause, H.M., Mäder, P., Fliessbach, A., Jarosch, K.A., Oberson, A., Mayer, J., 2024. Organic cropping systems balance environmental impacts and agricultural production. Scientific Reports 14, 25537. https://doi.org/10.1038/s41598-024-76776-1.

Krause, H.M., Stehle, B., Mayer, J., Mayer, M., Steffens, M., Mäder, P., Fliessbach, A., 2022. Biological soil quality and soil organic carbon change in biodynamic, organic, and conventional farming systems after 42 years. Agronomy for Sustainable Development 42, 117. https://doi.org/10.1007/s13593-022-00843-y.

Krause, H.-M., Thonar, C., Eschenbach, W., Well, R., Mäder, P., Behrens, S., Kappler, A., Gattinger, A., 2017. Long term farming systems affect soils potential for N2O production and reduction processes under denitrifying conditions. Soil Biology and Biochemistry 114. https://doi.org/10.1016/j.soilbio.2017.06.025.

Kundel, D., Bodenhausen, N., Jørgensen, H.B., Truu, J., Birkhofer, K., Hedlund, K., Mäder, P., Fliessbach, A., 2020. Effects of simulated drought on biological soil quality, microbial diversity and yields under long-term conventional and organic agriculture. FEMS Microbiology Ecology 96, 1–16. https://doi.org/10.1093/femsec/fiaa205.

Langmead, B., Salzberg, S.L., 2012. Fast gapped-read alignment with Bowtie 2. Nature Methods 9, 357–359. https://doi.org/10.1038/nmeth.1923.

Lehmann, J., Bossio, D.A., Kögel-Knabner, I., Rillig, M.C., 2020. The concept and future prospects of soil health. Nature Reviews Earth & Environment 1, 544–553. https://doi.org/10.1038/s43017-020-0080-8.

Levasseur, A., Drula, E., Lombard, V., Coutinho, P.M., Henrissat, B., 2013. Expansion of the enzymatic repertoire of the CAZy database to integrate auxiliary redox enzymes. Biotechnology for Biofuels 6, 1–14. https://doi.org/10.1186/1754-6834-6-41.

Li, D., Liu, C.M., Luo, R., Sadakane, K., Lam, T.W., 2015. MEGAHIT: An ultra-fast single-node solution for large and complex metagenomics assembly via succinct de Bruijn graph. Bioinformatics 31, 1674–1676. https://doi.org/10.1093/bioinformatics/btv033.

Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., Durbin, R., 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25, 2078–2079. https://doi.org/10.1093/bioinformatics/btp352.

Lori, M., Hartmann, M., Kundel, D., Mayer, J., Mueller, R.C., Mäder, P., Krause, H.M., 2023. Soil microbial communities are sensitive to differences in fertilization intensity in organic and conventional farming systems. FEMS Microbiology Ecology 99, 1–13. https://doi.org/10.1093/femsec/fiad046.

Louca, S., Polz, M.F., Mazel, F., Albright, M.B.N., Huber, J.A., O'Connor, M.I., Ackermann, M., Hahn, A.S., Srivastava, D.S., Crowe, S.A., Doebeli, M., Parfrey, L.W., 2018. Function and functional redundancy in microbial systems. Nature Ecology and Evolution 2, 936–943. https://doi.org/10.1038/s41559-018-0519-1.

Mäder, P., Fließbach, A., Dubois, D., Gunst, L., Fried, P., Niggli, U., 2002. Soil fertility and biodiversity in organic farming. Science 296, 1694–1697. https://doi.org/10.1126/science.1071148.

Martens-Habbena, W., Berube, P.M., Urakawa, H., De La Torre, J.R., Stahl, D.A., 2009. Ammonia oxidation kinetics determine niche separation of nitrifying Archaea and Bacteria. Nature 461, 976–979. https://doi.org/10.1038/nature08465.

Mayer, M., Krause, H.M., Fliessbach, A., Mäder, P., Steffens, M., 2022. Fertilizer quality and labile soil organic matter fractions are vital for organic carbon sequestration in temperate arable soils within a long-term trial in Switzerland. Geoderma 426. https://doi.org/10.1016/j.geoderma.2022.116080.

McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8. https://doi.org/10.1371/journal.pone.0061217.

Oberson, A., Frossard, E., 2015. Phosphorus management for organic agriculture. Phosphorus: Agriculture and Environment 46, 761–779. https://doi.org/10.2134/agronmonogr46.c24.

Oberson, A., Frossard, E., Bühlmann, C., Mayer, J., Mäder, P., Lüscher, A., 2013. Nitrogen fixation and transfer in grass-clover leys under organic and conventional cropping systems. Plant and Soil 371, 237–255. https://doi.org/10.1007/s11104-013-1666-4.

Oberson, A., Jarosch, K.A., Frossard, E., Hammelehle, A., Fliessbach, A., Mäder, P., Mayer, J., 2024. Higher than expected: nitrogen flows, budgets, and use efficiencies over 35 years of organic and conventional cropping. Agriculture, Ecosystems & Environment 362, 108802. https://doi.org/10.1016/j.agee.2023.108802.

Oberson, A., Nanzer, S., Bosshard, C., Dubois, D., Mäder, P., Frossard, E., 2007. Symbiotic N2 fixation by soybean in organic and conventional cropping systems estimated by 15N dilution and 15N natural abundance. Plant and Soil 290, 69–83. https://doi.org/10.1007/s11104-006-9122-3.

Ogawa, K., Akagawa, E., Yamane, K., Sun, Z.W., LaCelle, M., Zuber, P., Nakano, M.M., 1995. The nasB operon and nasA gene are required for nitrate/nitrite assimilation in Bacillus subtilis. Journal of Bacteriology 177, 1409–1413. https://doi.org/10.1128/jb.177.5.1409-1413.1995.

Oksanen, A.J., Blanchet, F.G., Kindt, R., Legen, P., Minchin, P.R., Hara, R.B.O., Simpson, G.L., Solymos, P., Stevens, M.H.H., 2012. Vegan: community ecology package. http://CRAN.R-project.org/package=vegan.

O'Leary, N.A., Wright, M.W., Brister, J.R., Ciufo, S., Haddad, D., McVeigh, R., Rajput, B., Robbertse, B., Smith-White, B., Ako-Adjei, D., Astashyn, A., Badretdin, A., Bao, Y., Blinkova, O., Brover, V., Chetvernin, V., Choi, J., Cox, E., Ermolaeva, O., Farrell, C. M., Goldfarb, T., Gupta, T., Haft, D., Hatcher, E., Hlavina, W., Joardar, V.S., Kodali, V.K., Li, W., Maglott, D., Masterson, P., McGarvey, K.M., Murphy, M.R., O'Neill, K., Pujar, S., Rangwala, S.H., Rausch, D., Riddick, L.D., Schoch, C., Shkeda, A., Storz, S.S., Sun, H., Thibaud-Nissen, F., Tolstoy, I., Tully, R.E., Vatsan, A. R., Wallin, C., Webb, D., Wu, W., Landrum, M.J., Kimchi, A., Tatusova, T., DiCuccio, M., Kitts, P., Murphy, T.D., Pruitt, K.D., 2016. Reference sequence (RefSeq) database at NCBI: current status, taxonomic expansion, and functional annotation. Nucleic Acids Research 44, D733–D745. https://doi.org/10.1093/nar/gkv1189.

Orellana, L.H., Chee-Sanford, J.C., Sanford, R.A., Löffler, F.E., Konstantinidis, K.T., 2018. Year-round shotgun metagenomes reveal stable microbial communities in agricultural soils and novel ammonia oxidizers responding to fertilization. Applied and Environmental Microbiology 84, 1–14. https://doi.org/10.1128/AEM.01646-17.

Overbeek, R., Olson, R., Pusch, G.D., Olsen, G.J., Davis, J.J., Disz, T., Edwards, R.A., Gerdes, S., Parrello, B., Shukla, M., Vonstein, V., Wattam, A.R., Xia, F., Stevens, R., 2014. The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). Nucleic Acids Research 42, 206–214. https://doi.org/10.1093/nar/gkt1226.

Potapov, P., Turubanova, S., Hansen, M.C., Tyukavina, A., Zalles, V., Khan, A., Song, X., Pickens, A., Shen, Q., Cortez, J., 2022. Global maps of cropland extent and change show accelerated cropland expansion in the twenty-first century. Nature Food 3, 19–28. https://doi.org/10.1038/s43016-021-00429-z.

Reimer, M., Hartmann, T.E., Oelofse, M., Magid, J., Bünemann, E.K., Möller, K., 2020. Reliance on biological nitrogen fixation depletes soil phosphorus and potassium reserves. Nutrient Cycling in Agroecosystems 118, 273–291. https://doi.org/10.1007/s10705-020-10101-w.

Rognes, T., Flouri, T., Nichols, B., Quince, C., Mahé, F., 2016. VSEARCH: a versatile open source tool for metagenomics. PeerJ 2016, 1–22. https://doi.org/10.7717/peerj.2584.

Schmieder, R., Edwards, R., 2011. Quality control and preprocessing of metagenomic datasets. Bioinformatics 27, 863–864. https://doi.org/10.1093/bioinformatics/btr026.

Seufert, V., Ramankutty, N., 2017. Many shades of gray—the context-dependent performance of organic agriculture. Science Advances 3, e1602638. https://doi.org/10.1126/sciadv.1602638.

Seufert, V., Ramankutty, N., Foley, J.A., 2012. Comparing the yields of organic and conventional agriculture. Nature 485, 229–232. https://doi.org/10.1038/nature11069.

Shannon, P., Markiel, A., Owen, O., Baliga, N.S., Wang, J.T., Ramage, D., Amin, N., Schwikowski, B., Ideker, T., 2003. Cytoscape: a software environment for integrated models. Genome Research 13, 426. https://doi.org/10.1101/gr.1239303.

Skinner, C., Gattinger, A., Krauss, M., Krause, H.-M., Mayer, J., van der Heijden, M.G.A., Mäder, P., 2019. The impact of long-term organic farming on soil-derived greenhouse gas emissions. Scientific Reports 9, 1702. https://doi.org/10.1038/s41598-018-38207-w.

Storey, J.D., Bass, A.J., 2023. Bioconductor's Qvalue Package. https://doi.org/10.18129/B9.bioc.qvalue.

Storey, J.D., Tibshirani, R., 2003. Statistical significance for genomewide studies. Proceedings of the National Academy of Sciences 100, 9440–9445. https://doi.org/10.1073/pnas.1530509100.

Tilman, D., Balzer, C., Hill, J., Befort, B.L., 2011. Global food demand and the sustainable intensification of agriculture. Proceedings of the National Academy of Sciences 108, 20260–20264. https://doi.org/10.1073/pnas.1116437108.

Torres-Guzman, J.C., Padilla-Guerrero, I.E., Cervantes-Quintero, K.Y., Martinez-Vazquez, A., Ibarra-Guzman, M., Gonzalez-Hernandez, G.A., 2021. Peculiarities of nitronate monooxygenases and perspectives for in vivo and in vitro applications. Applied Microbiology and Biotechnology 105, 8019–8032. https://doi.org/10.1007/s00253-021-11623-1.

Tu, Q., Lin, L., Cheng, L., Deng, Y., He, Z., 2019. NCycDB: a curated integrative database for fast and accurate metagenomic profiling of nitrogen cycling genes. Bioinformatics 35, 1040–1048. https://doi.org/10.1093/bioinformatics/bty741.

Vocadlo, D.J., Davies, G.J., 2008. Mechanistic insights into glycosidase chemistry. Current Opinion in Chemical Biology 12, 539–555. https://doi.org/10.1016/j.cbpa.2008.05.010.

Wickham Hadley, 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag, New York. ISBN 978-3-319-24277-4. doi:ggplot2.tidyverse.org.

Wu, X., Cui, Z., Peng, J., Zhang, F., Liesack, W., 2022. Genome-resolved metagenomics identifies the particular genetic traits of phosphate-solubilizing bacteria in agricultural soil. ISME Communications 2, 27–30. https://doi.org/10.1038/s43705-022-00100-z.

Xu, J., Kim, J., Danhorn, T., Merritt, P.M., Fuqua, C., 2012. Phosphorus limitation increases attachment in Agrobacterium tumefaciens and reveals a conditional functional redundancy in adhesin biosynthesis. Research in Microbiology 163, 674–684. https://doi.org/10.1016/j.resmic.2012.10.013.

Zeng, J., Tu, Q., Yu, X., Qian, L., Wang, C., Shu, L., Liu, F., Liu, S., Huang, Z., He, J., Yan, Q., He, Z., 2022. PCycDB: a comprehensive and accurate database for fast analysis of phosphorus cycling genes. Microbiome 10, 1–16. https://doi.org/10.1186/s40168-022-01292-1.

Zhang, X., Zou, T., Lassaletta, L., Mueller, N.D., Tubiello, F.N., Lisk, M.D., Lu, C., Conant, R.T., Dorich, C.D., Gerber, J., Tian, H., Bruulsema, T., Maaz, T.M.C., Nishina, K., Bodirsky, B.L., Popp, A., Bouwman, L., Beusen, A., Chang, J., Havlík, P., Leclère, D., Canadell, J.G., Jackson, R.B., Heffer, P., Wanner, N., Zhang, W., Davidson, E.A., 2021. Quantification of global and national nitrogen budgets for crop production. Nature Food 2, 529–540. https://doi.org/10.1038/s43016-021-00318-5.

Zuckerberg, C., 2022. czid-dedup. github.com/chanzuckerberg/czid-dedup.