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Land use drives prokaryotic community composition of directly adjacent grasslands

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Abstract
Understanding the impact of agricultural land use on the soil prokaryotic communities in connected downslope sites is crucial for developing sustainable strategies to preserve ecosystem properties and mitigate agriculture's environmental impacts. In this study, we investigated topsoil samples collected at three time points in 2022 (March, June, and November) from two adjacent catenas, reaching from hillslope to floodplain. The catenas differed in land use (extensive grassland vs. extensive cropland) at the top and middle parts, while the floodplain remained an extensive grassland due to legal restrictions. Using quantitative real-time PCRs and metabarcoding, we assessed prokaryotic abundance and prokaryotic community composition. Results show higher bacterial abundance in the cropland-influenced floodplain part across all time points compared to the grassland-influenced floodplain part. Temporal dynamics revealed a progressive decrease in the shared prokaryotic communities of the floodplain parts, peaking at the summer sampling time point, indicating a significant influence of the respective management type of the agricultural sites over the bacterial and archaeal communities of the floodplain parts. Differential abundance analyses identified several nitrifying taxa as more abundant in the cropland-influenced floodplain. Upstream land use also influenced the prokaryotic network of the cropland-floodplain, with some cropland taxa becoming keystone taxa and altering network morphology, an effect not observed in the grassland-influenced floodplain. These findings suggest that upstream agricultural land use practices have exerted a long-term influence on the floodplain prokaryotic communities over the past three decades. Moreover, there is evidence suggesting that these prokaryotic communities may undergo a potential reset during winter, which requires further investigation.

Keywords 16S rRNA gene amplicon sequencing · Prokaryotic diversity · Land use · Temperate grassland · Prokaryotic networks

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Introduction

Temperate grasslands store one third of the terrestrial soil organic carbon (SOC) and play an important role in biodiversity conservation. In addition, they provide a wide range of further soil functions and ecosystem services including water regulation by soil water infiltration and evapotranspiration, and nutrient regulation as well as groundwater protection through water filtration function (Bengtsson et al. 2019; Sirimarco et al. 2018). As global food demand increases, many of these sites have been transformed into agricultural land in the last decades to be used for crops production (Wu et al. 2018). It is well documented that such land use changes can disrupt carbon sequestration, entail soil erosion, hinder productivity, worsen water quality and reduce biodiversity by fragmentation or alteration of native vegetation (Chowdhury et al. 2021; de Snoo et al. 2012; Li et al. 2020; Vanwalleghem et al. 2017). In addition, sites which are still used as grasslands are progressively receiving an impact due to agricultural management as fertilizer-derived nutrients and pesticides run-off (Hou et al. 2021; Zajíček et al. 2018).

While the consequences of land use change of grassland sites into crop production areas have been extensively studied, the direct impact of sites under crop production on adjacent downslope floodplain grasslands is still poorly understood. In particular, if this impact might be mainly driven by nutrient runoff, microbiological influence, or other factors, such as soil erosion or water management practices. This particular case is highly relevant given that these riparian areas sustain high diversity levels and provide essential ecosystem services, including water and nutrient retention (Jakubínský et al. 2021; Opperman et al. 2010).

In grasslands under extensive management, the soil microbiome plays a key role in productivity and filtering function, as microorganisms catalyse nutrient turnover and immobilization, and regulate plant nutrient uptake, as well as belowground plant interactions (Giller et al. 1997; Jansson and Hofmockel 2018; Van Der Heijden et al. 2008; Wang et al. 2019a). Microbial diversity can also contribute to increase the ecosystems resistance and resilience towards perturbations (Girvan et al. 2005). These properties are especially pertinent given the context of climate change, which might increase the frequency, severity and duration of drought and other extreme weather events in temperate regions (Bardgett and Caruso 2020; Stott 2016). Therefore, it is of prime importance to protect the soil microbiome and its diversity for a sustainable preservation of grassland sites and their environmental functions (Bardgett and Van der Putten 2014).

The diversity, composition, and function of soil microbiomes can be influenced by several factors, including soil properties, climate, land management practices, and plant diversity (Delgado-Baquerizo et al. 2018; Hartman et al. 2018; Philippot et al. 2013). Land-use changes, such as grassland-to-cropland conversion, have been shown to alter microbial communities, with an increase in potentially pathogenic taxa in crop production sites (French et al. 2017). This transition can also increment *Gemmatimonadetes* abundance while reducing *Verrucomicrobia* and *Planctomycetes* (Szoboszlay et al. 2017).

Given the above, it can be expected that cropping systems located uphill from natural floodplain grasslands may have a strong impact on the composition and function of downstream prokaryotic communities of such sites, as a source of nutrients and pesticides as well as microbiota. Such changes would have subsequent effects in nutrient cycling, soil structure, plant health and productivity given the critical roles of soil prokaryotic communities in these processes. However, data confirming this hypothesis is still missing as the composition of prokaryotic communities along downstream sites under different land use types has not been yet thoroughly investigated in temperate grasslands.

To address this gap of knowledge, we examined the influence of an upslope extensively managed grassland versus an extensively managed upslope cropland on the soil prokaryotic communities of a connected temperate grassland floodplain. The assessment was made by comparing the soil prokaryotic communities of the differently influenced parts of the floodplain, which had a similar plant species composition. To study the prokaryotic community composition, we used a molecular metabarcoding approach which was complemented by quantitative real time PCR (qPCR) measurements to determine the abundance of prokaryotes.

Materials and methods

Experimental site and sample collection

The study was carried out in Süssenbach (Bavaria, Germany; 49º06'13" N 12º21'28" E – 473 m). The area receives an annual mean precipitation of 875 mm and has an annual mean temperature of 8.9 °C. The study site had an overall size of 3.35 ha and is comprising a creek (Otterbach) with all floodplains used as grasslands and riparian buffer zones, which are characterized by soils with a loamy texture, and adjacent slopes with either a cropland or grassland use. The current landowners have been managing the site since 1995, and its appearance has remained largely unchanged since then. The presence of Ap horizons and terraces suggests that the site was likely used for crop production in earlier centuries.

We focused on two pairs of sites in the study area, including a site used for cropping (C) and the downslope floodplain part (FP-C), as well as an extensively managed grassland (G) and its downslope floodplain part (FP-G). Both floodplain sites had a similar plant species composition, dominated by *Alopecurus pratensis*, *Anthoxanthum odoratum* and *Veronica chamaedrys*, while at the extensively managed grassland *Dactylis glomerata*, *Alopecurus pratensis* and *Plantago lanceolata* were more abundant. The extensively and organically managed grassland was mowed once a year at the end of May. The cropland, also managed organically, featured a diverse cropping sequence comprising oat (*Avena sativa*), triticale (*Tritico secale*), winter barley (*Hordeum vulgare*), and rye (*Secale cereale*), the third of which was cultivated at the beginning of the experiment. It was initially sown in October 2021 and harvested at the end of June 2022. Afterwards, rye was cultivated at the site. Tillage was performed before the cultivation of winter barley and rye up to a depth of 25 cm. Both the cropland and the grassland were fertilised with 15 m^3 per hectare of nitrogen-rich slurry (liquid manure) in March 2022 and October 2022 using a drag hose system, and liming was conducted on both the cropland and the grassland. The slurry was analyzed by a commercial laboratory (Raiffeisen-Laborservice, Ormont, Germany) and was found to contain an average of 2.61 kg m^{-3} total nitrogen. Of this, 45.6% (1.19 kg m^{-3}) was in the form of ammonium, while the remaining 54.4% (1.42 kg m^{-3}) was organic nitrogen, including urea (Sebastian Floßmann 2024, personal communication).

The pH of all sites was comparable and ranged from 5.5 for soil samples from the two floodplain sites, to 6.0 for the soil samples from the cropland. As a matter of the management, sites differed in their total carbon (TC) and nitrogen (TN), whose contents were determined with an Elemental Analyzer (Euro EA, Eurovector, Milano, Italy). Higher values were found in soil samples from the grassland (5.08% ± 0.8 and 0.43% ± 0.06). In contrast, the lowest values were found in the cropland samples (2.44 ± 0.2 and 0.22% ± 0.018, respectively). Soil samples from both floodplains were comparable in TC and TN (3.90% ± 0.78 and 0.34% ± 0.06 for FP-G, and 4.27% ± 0.26 and 0.36% ± 0.03 for FP-C, respectively) (Table S1).

Bulk soil samples from 0 to 5 cm were collected at three sampling times in 2022 (March, June and November) along the two catenas following the design and specifications displayed in Fig. 1b using a soil auger with a diameter of 15 cm. Both catenas included three sampling points at the extensively managed grassland or the cropland and one at the floodplains, just before the Otterbach River. Five soil replicates separated by 1 m were collected per sampling point resulting in an overall of 120 samples (3 sampling time points * 8 sampling points * 5 replicates per sampling point). Samples were homogenised with a 4 mm sieve and stored at -80ºC for molecular analyses. Soil water content was determined by drying soil to a constant weight at 65 °C for 48 h (Table S2).

DNA extraction

Genomic DNA was extracted from 0.25 g of soil using the NucleoSpin® Soil Kit (Macherey-Nagel, Düren, Germany) following the manufacturer's guidelines. The SL1 buffer was used for the chemical cell lysis, and the Precellys24 homogenizer (Bertin Technologies, France) was used for the mechanical cell lysis. The extracted DNA was quantified, and quality checked using the Quant-iT PicoGreen dsDNA Assay Kit (Thermo Fisher Scientific, Darmstadt, Germany). Bead beating tubes with all reactants but without soil were used as negative extraction controls. 50 µL of

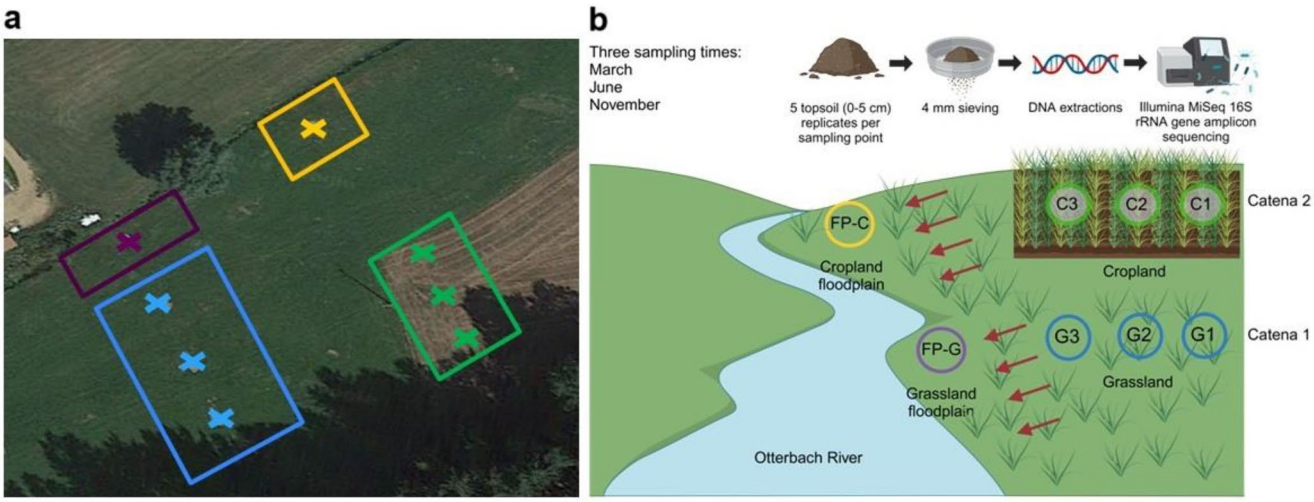

Fig. 1 **a** Aerial view of the experimental site in Süssenbach (Germany), obtained from Google Earth, and sampling points. **b** Experimental design

diethyl pyrocarbonate (DEPC) water was used to elute the extracted DNA.

Quantitative real time PCR (qPCR)

The 16 S rRNA gene was targeted to measure both bacterial and archaeal biomass in the soil samples. SYBR Green-based (Applied Biosystems, Warrington, UK) qPCR assays were performed on a 7300 Real-time PCR System (Thermo Fisher Scientific, Darmstadt, Germany) using the FP16S and RP16S primers (Bach et al. 2002) for bacteria, and the rSAf(i) (Nicol et al. 2003) and 958r (Bano et al. 2004) primers for archaea. The deployed standards are indicated in Table S3, as well as previously described parameters and conditions (Chiba et al. 2021). The optimal dilution rate of DNA extracts was fixed to 1:32 after testing for PCR inhibition with a serial dilutions test (data not shown). PCR reaction mixtures (25 µL) contained 12.5 µL of SYBR Green PCR Master Mix (Thermo Fisher Scientific, Darmstadt, Germany), 10 pmol of each primer, 8.5 µL of DEPC water, and 2 µL of DNA template. 2 µL of DEPC water and the mentioned reagents were used to constitute the assay's negatives. The amplification efficiency of the qPCR runs exceeded 90%, while the R^2 value of the standard curves exceeded 0.99. Quality and size of the qPCR products were electrophoretically checked using 1% (w/v) agarose gels and a dissociation-curve analysis, which was performed with the 7300 System SDS Software v1.3.0 (Applied Biosystems).

Amplicon sequencing

For the determination of the prokaryotic community composition, we followed the quality guidelines of the 16 S rRNA Metagenomic Sequencing Library Preparation protocol (Illumina, San Diego, CA, USA) and Schöler et al. (2017), in which DNA extracts were used for the amplification of the V4 region of the 16 S rRNA gene using the improved Earth Microbiome project primers for bacteria and archaea 515 F (Parada et al. 2016) and 806R (Apprill et al. 2015). More information about the library preparation can be found in the Supplementary Material.

Bioinformatic processing

The removal of sequencing adapters was performed using "fastp" v0.12.4 (Chen et al. 2018). Subsequently, sequenced reads underwent processing using the "DADA2" v1.22.0 pipeline (Callahan et al. 2016), following the protocol outlined in (https://benjjneb.github.io/dada2/tutorial.html). Briefly, sequencing primers were removed, and reads were quality filtered using a minimum length of 245 and 189 nucleotides for forward and reverse reads, respectively. A minimum quality score of 28 and a maximum number of allowed errors of 2 and 4 for forward and reverse reads, respectively, were established with the function "maxEE". The resulting sequences were treated as amplicon sequence variants (ASVs), and their nucleotide sequences and abundances were exported to "QIIME2" v2-2022.08 (Bolyen et al. 2018) as representative sequence artefacts and feature table artefact for taxonomic classification. A more thorough description of the processing with "QIIME2" can be found in the Supplementary Material. The output of "QIIME2" was exported into a "phyloseq" v1.38.0 (McMurdie and Holmes 2013) object using the package "qiime2R" v0.99.6 (Bisanz 2018). 15 ASVs from the negative controls were classified as contaminants when establishing a prevalence threshold of 0.05 and 0.1 in the samples and were subsequently removed using the "decontam" v1.12 package (Davis et al. 2018). Mitochondrial and chloroplast sequences were removed from the dataset using the "subset_taxa" function of "phyloseq". The rarefaction curves of the samples were plotted with the "rarecurve" function of the "vegan" v2.6-4 (Oksanen et al. 2013) package. Rarefaction curves indicated that the sequencing depth per sample was sufficient to cover the complete prokaryotic diversity in the samples (Fig. S1). The dataset was normalised with the Cumulative Sum Scaling (CSS) method (Paulson et al. 2013) using the "normalize" function of the "microbiomeMarker" v1.0.2 package (Cao et al. 2022).

Statistical analyses

The alpha diversity indices Chao, Shannon, and the observed ASVs number were estimated using the "alpha" function of the "microbiome" v.1.22 (Lahti and Shetty 2018) package. Differences regarding observed ASVs number and 16 S rRNA gene copies per g^{-1} of dry weight between sampling points at the same catena level and sampling time were calculated and included in the plots using Wilcoxon rank sum tests within the "stat_compare_means" function of the "ggpubr" v0.6.0 package (Kassambara 2023).

The spatial ordination of the samples at each sampling time was estimated using principal coordinate analysis (PCoA) and the Bray-Curtis dissimilarity index (Bray and Curtis 1957) using the "plot_ordination" function of the "phyloseq" package. Compositional differences between sites were calculated with the permutational multivariate analysis of variance (PERMANOVA) within the "adonis2" function of the "vegan" package using 999 permutations.

Differential abundance analyses comparing cropland and grassland samples and the two floodplains at the three sampling times were carried out with the edgeR method using the "run_edger" function of the "microbiomeMarker" package, which is based on the negative binomial distribution.

Significance was set to -2 and 2 log2FoldChange for FP-C and FP-G, respectively, the *P*-value cut-off was fixed to 0.001 and the false discovery rate (fdr) was used as multiple testing adjustment method.

Moreover, core prokaryotic community analyses were carried out with the "core_members" function of the "microbiome" package and plotted with the "ggvenn" v0.1.10 (Yan 2021) package.

The R package "NetCoMi" v.1.1.0 (Peschel et al. 2021) was used for the prokaryotic network analyses using the Spearman coefficient as co-association measure, centered log-ratio (clr) transformation as normalization method, local fdr as multiple testing adjustment method, t-test as the sparsification method and a significance level of 0.001. Bacterial and archaeal community composition differences across floodplains were estimated using the "cluster_fast_greedy" algorithm (Clauset et al. 2004).

Results

Quantification of bacterial and archaeal abundance

16 S rRNA gene copies per g^{-1} dry soil measured by qPCR were used as a proxy to assess differences in bacterial (Fig. 2a) and archaeal (Fig. 2b) abundance. Bacterial abundance was lower in the soil samples from the cropland than from the grassland in March (1.51×10^{10} and 1.71×10^{10} copies per g^{-1} dry soil, respectively), while the opposite was observed in June (2.1×10^{10} and 6.75×10^{9} copies per g^{-1} dry soil, respectively) and November (9.95×10^{9} and 4.75×10^{9} copies per g^{-1} dry soil, respectively). Differences were significant in June when comparing G1 with C1, and G3 with C3 ($P<.05$; Table S4), and in November when comparing G1 with C1, and G2 with C2 ($P<.05$; Table S4). Regarding the floodplain sites, soil samples from FP-C were higher in bacterial abundance than FP-G in March (2.36×10^{10} and 1.68×10^{10} copies per g^{-1} dry soil,

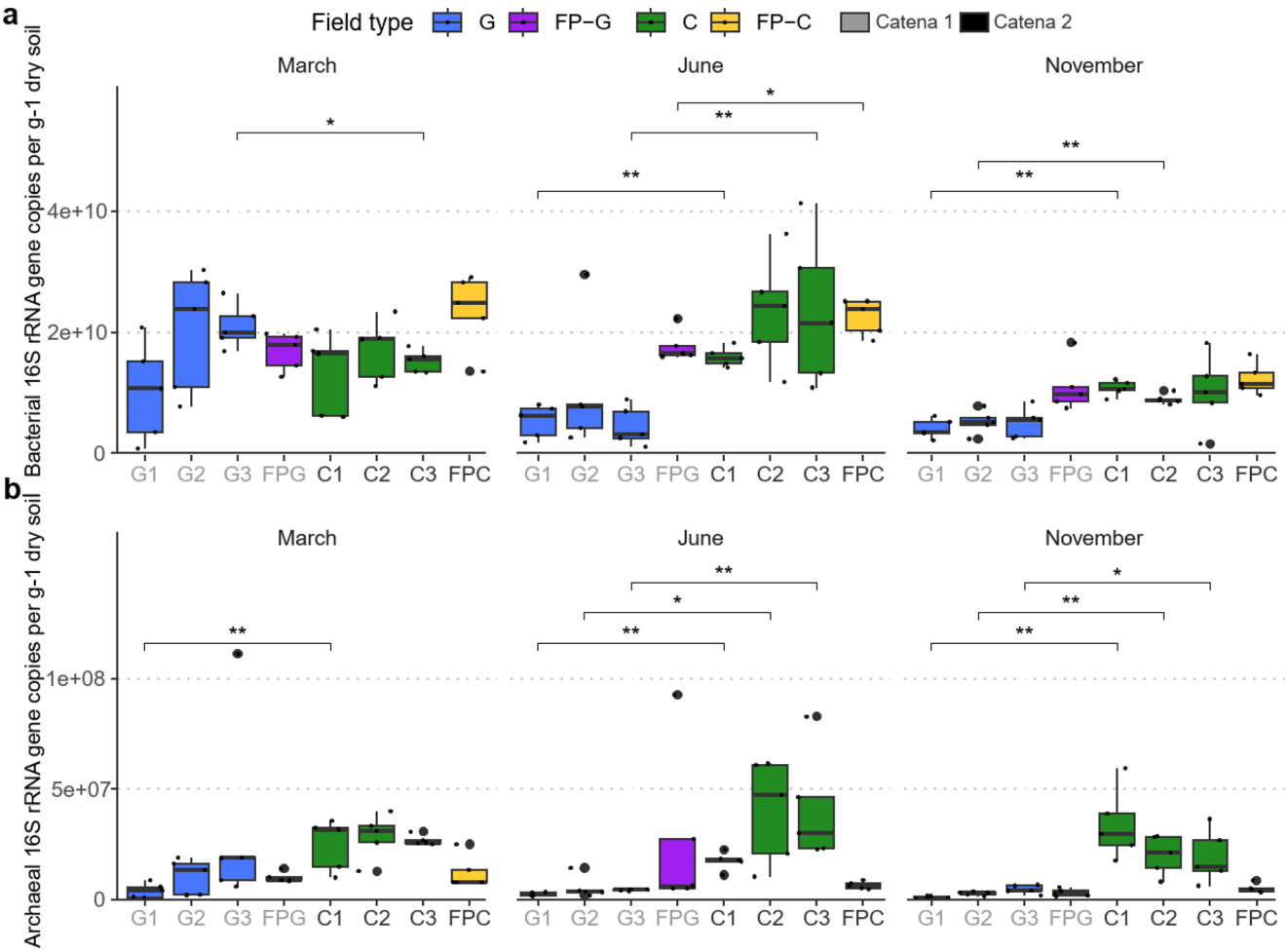

Fig. 2 Number of 16S rRNA gene copies per gram of dry soil detected with the qPCR reaction at each of the sampling times for **a** bacteria and **b** archaea. Differences between sampling points at the same catena level and sampling time were calculated using the Wilcoxon rank sum test. *P*-values denoted as **<0.01, *<0.05, ns not shown

respectively), June (2.26×10^{10} and 1.77×10^{10} copies per g^{-1} dry soil, respectively) and November (1.23×10^{10} and 1.1×10^{10} copies per g^{-1} dry soil, respectively), however, these differences were only statistically significant in June ($P<.05$; Table S4).

Archaeal abundance was higher in the soil samples from the cropland when compared to the grassland in March (2.68×10^{7} and 1.57×10^{7} copies per g^{-1} dry soil, respectively), June (3.29 x 10^{7} and 4.07×10^{6} copies per g-1 dry soil, respectively) and November (2.46×10^{7} and 2.80×10^{6} copies per g^{-1} dry soil, respectively). Differences were significant in March only when comparing G1 with C1 ($P<.01$; Table S4), in June when comparing G1 with C1 ($P<.01$), G2 with C2 ($P<.05$), and G3 with C3 ($P<.01$; Table S4). In November differences were significant when comparing G1 with C1 ($P<.01$), G2 with C2 ($P<.01$) and G3 with C3 ($P<.05$; Table S4). Regarding the floodplains, soil samples from FP-C had a higher archaeal biomass than FP-G in March (1.24×10^{7} and 9.96×10^{6} copies per g^{-1} dry soil, respectively) and November (5×10^{6} and 3.10×10^{6} copies per g^{-1} dry soil, respectively), but not in June (6.47×10^{6} and 2.73×10^{7} copies per g^{-1} dry soil, respectively). However, these differences were not significant at any sampling time (Table S4).

Prokaryotic alpha and beta diversity

15,829,439 paired end reads of the amplified V4 region of the 16 S rRNA gene were obtained across 120 samples, of which, 8,047,043 sequences remained after the filtering steps (Table S5) and resulted in 162,470 ASVs after the taxonomic assignment.

Richness expressed as the observed ASVs number was used as a proxy for prokaryotic alpha diversity (Fig. 3a). Richness was generally lowest in November, especially in FP-C and G2. However, no significant richness differences were found when comparing sampling points at the same catena level and sampling time (Table S6). Other alpha

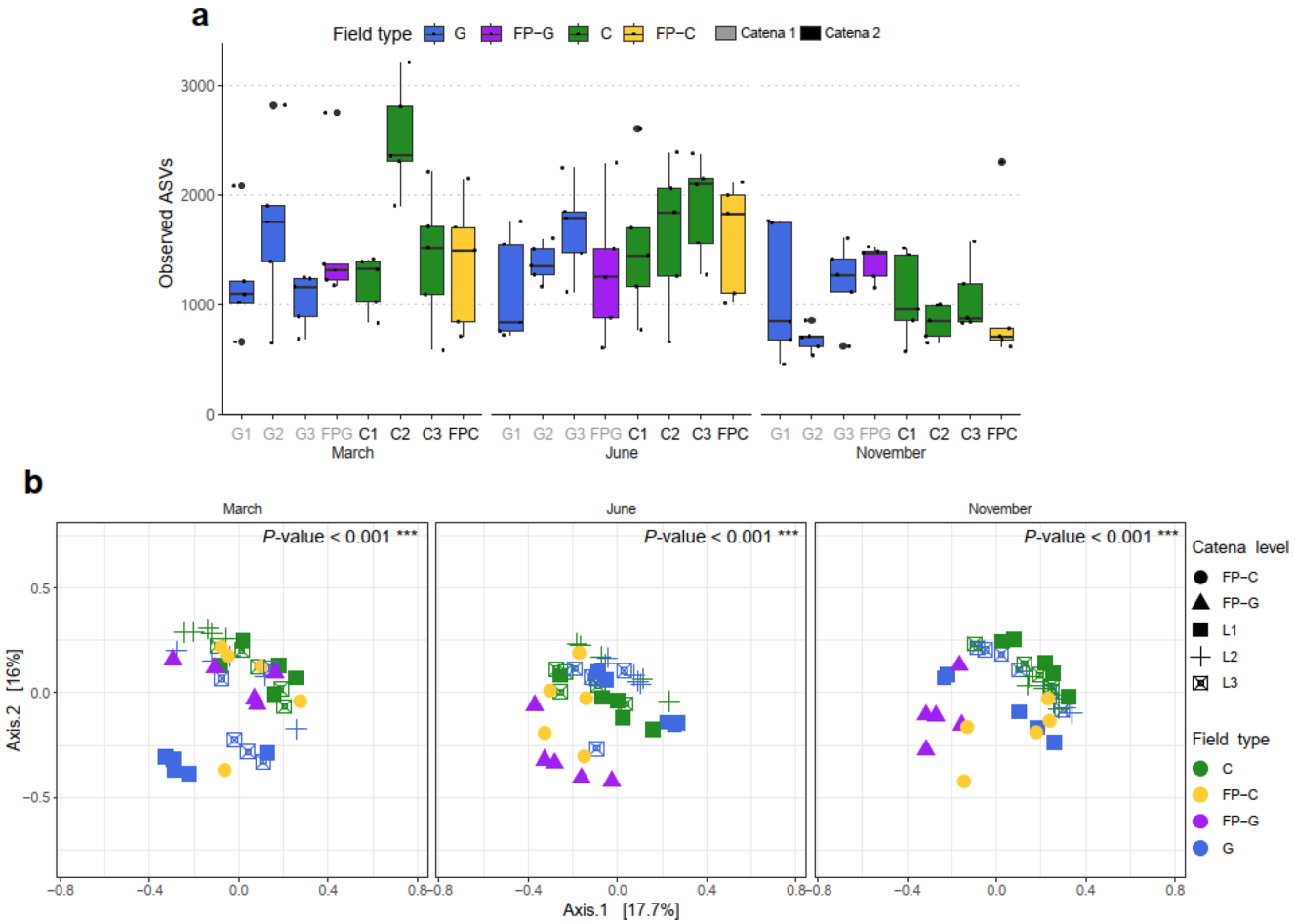

Fig. 3 **a** Alpha diversity expressed as richness (number of observed ASVs) across sampling points and times. Differences between sampling points at the same catena level and sampling time were calculated using the Wilcoxon rank sum test. None of the obtained *P*-values were significant. **b** Principal coordinate analysis (PCoA) calculated with the Bray-Curtis dissimilarity at the three sampling times. Shapes depending on the catena level and colours based on the field type. Dissimilarity differences within field types were calculated using PERMANOVA tests

diversity indices like Chao and Shannon showed similar trends (Table S7).

A Principal coordinate analysis (PCoA) for each sampling timepoint was carried out to address beta diversity variations across the sites, using the Bray-Curtis distance to quantify the dissimilarity between samples (Fig. 3b). Bacterial and archaeal community composition was significantly different across the field types at the three sampling times (PERMANOVA, $P < .001$; Table S8).

When addressing treatment pairs' beta diversity differences at each of the three sampling times with Tukey's test (Table S9), only significant differences were found between the cropland and grassland, and the grassland and FP-G for March ($P < .05$ and $P < .05$, respectively). Nevertheless, the significance of the differences between FP-C and FP-G increased steadily throughout the year, with *P*-values rising from 0.873 in March to 0.057 in November. The heterogeneity of prokaryotic communities across the experimental sites was assessed through beta dispersion analyses, nevertheless no significant results were found (Fig.S2a, Fig. S2b and Fig. S2c).

Bacterial and archaeal community composition and differential abundance analyses

The number of prokaryotic taxa present in soil samples from all sites (core of bacterial and archaeal communities) decreased along the three sampling time points (175 ASVs 14.4%, 156 ASVs 11.3%, 96 ASVs 9% in March, June and November, respectively) as indicated by the Venn diagram (Fig. 4). At the same time, the shared ASVs of the cropland and FP-C (Fig. S3a) increased from March (381 ASVs 37.2%) to June (425 ASVs 40.5%) and then decreased in November again (116 ASVs 21.6%). Conversely, the number of shared ASVs from grassland and FP-G (Fig. S3b) decreased from March (244 ASVs 31.9%) to June (176 19%) and then recovered slightly in November (267 ASVs 29.4%). When investigating the stability of the prokaryotic communities' core of the three sampling points of the cropland (C1, C2 and C3) and the grassland (G1, G2 and G3) at the three sampling times (Fig. S4a and Fig. S4b, respectively), the cropland was as expected more stable, as the number of shared ASVs ranged from 67.4% in March to 69.9% in June, and to 63.5% in November, while for the grassland shared ASVs were in the range of 35.8% in March, 68.7% in June and 57.3% in November.

Regarding the number of site specific ASVs, when comparing the cropland and grassland sites, 600 (65.8%), 383 (34.8%) and 227 (33.7%) specific ASVs (Fig. S5a) were detected at the cropland, while for the grassland 84 (9.2%), 285 (25.9%) and 187 ASVs (27.8%) were detected in March, June and November, (Fig. S5a), respectively. When comparing FP-C and FP-G, FP-C 142 (16.9%), 400 (51.2%) and 13 (1.8%) specific ASVs were detected (Fig. S5b), while for FP-G 260 (31%), 124 (15.9%) and 574 (77.5%) specific ASVs for the same time points as described above (Fig. S5b).

When examining the bacterial and archaeal communities specific ASVs that differed between soil samples from the sites, the differential abundance analyses comparing the cropland and the grassland at each of the three sampling times (Fig. 5a), with the effect size estimated as Log2FoldChange, resulted in 27, 20 and 21 featured ASVs in the cropland for March, June and November. Of those, *Acidobacteriaceae*, *Geodermatophilaceae*, *Luteimonas*, *Nitrospira*, *Nitrosophaeria*, *Nitrosophaeraceae*, *Nostocaceae* or *Terrabacter* were consistently differentially abundant. In the grassland, 16, 6 and 8 ASVs were enriched in March, June and November. Of those, *Candidatus Xiphinematobacter* and *Paenibacillus* were consistent within sampling times. When the same analysis was performed comparing FP-C with FP-G (Fig. 5b), 8, 4 and 17 differentially abundant ASVs were detected in FP-C in March, June and November, including several ASVs assigned to the archaeal class *Nitrosophaeria*. For soils samples from FP-G, 5 and 18 ASVs were detected in March and June; interestingly, no ASVs were differentially abundant in November.

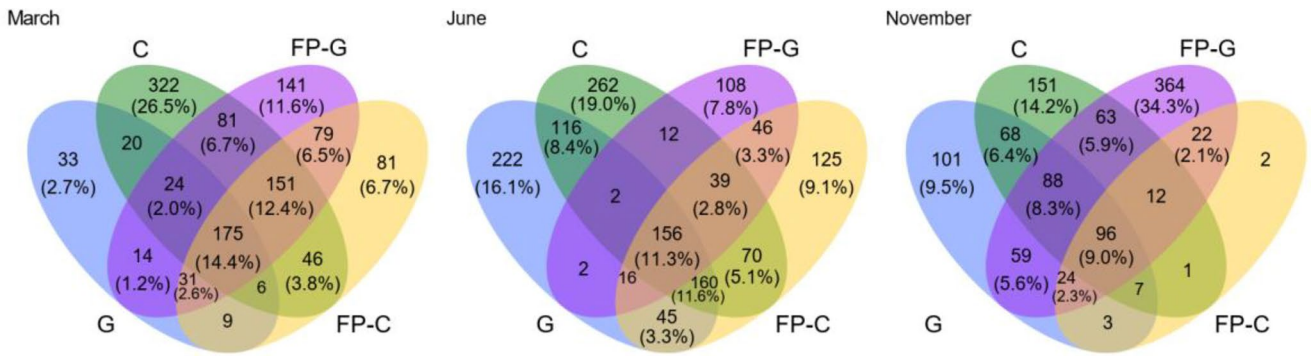

Fig. 4 Venn diagrams showing the number and relative abundance (%) of shared and site-specific ASVs between the four sites of the study: grassland (G), cropland (C), grassland floodplain (FP-G) and cropland floodplain (FP-C). Only relative abundances higher than 2% are shown

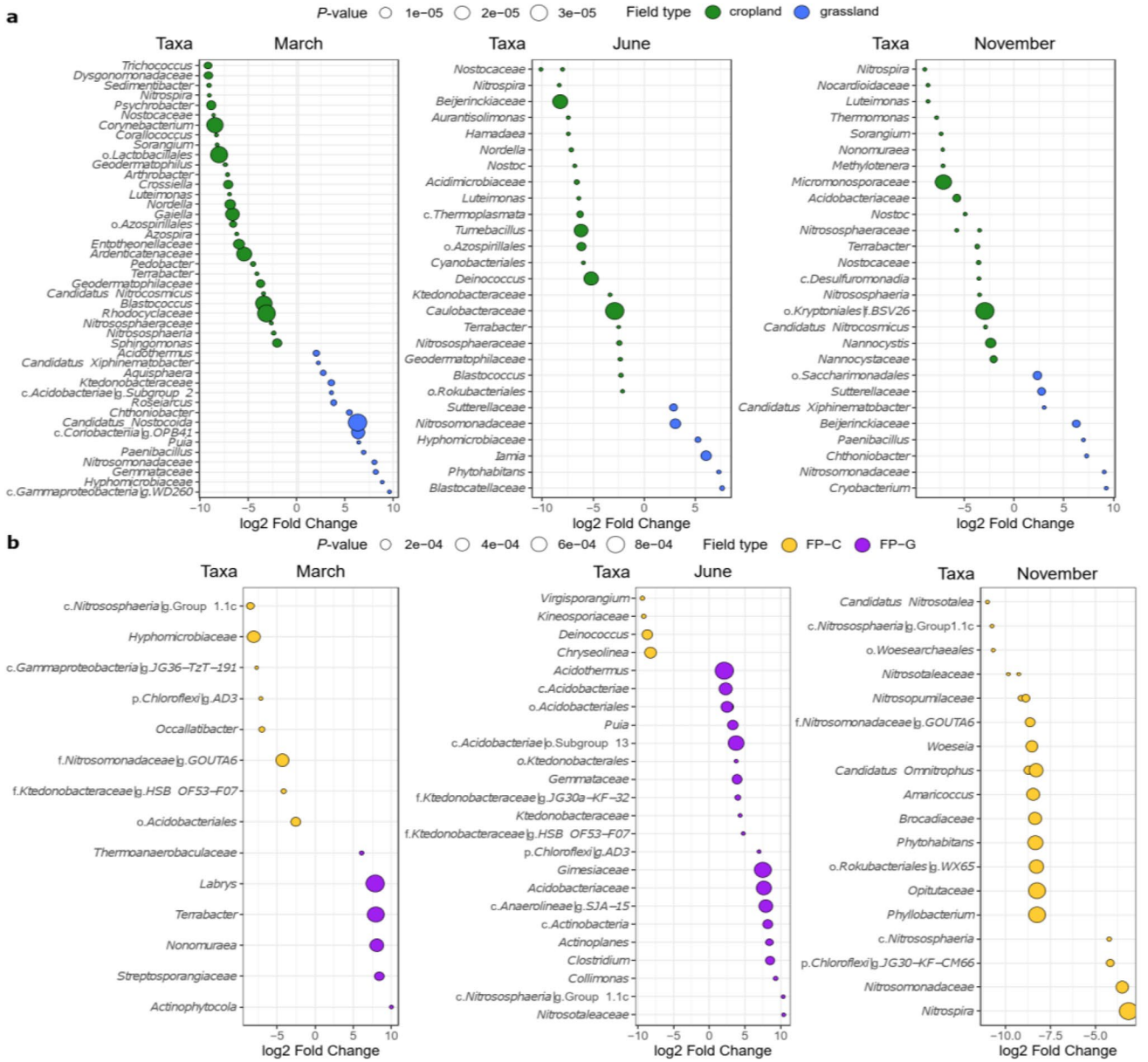

Fig. 5 Bubble plots representing the results of differential abundance analyses using edgeR at the three sampling times comparing: **a** cropland and grassland **b** cropland floodplain (FP-C) and grassland floodplain (FP-G). Differences were represented as log 2-Fold Change in the x axis (the threshold was set to -2 and 2 for cropland and grassland, and FP-C and FP-G, respectively). The size of the bubbles represents the P-value, whose cutoff was fixed to 0.05

Prokaryotic networks

The prokaryotic networks of the two floodplains showed substantial differences regarding most network properties. Results (Table 1) show a lower clustering coefficient, edge density and natural connectivity at FP-C (0.677, 0.161 and 0.22) when compared to FP-G (0.886, 0.467 and 0.537). FP-C showed a higher relative LCC size, modularity, dissimilarity and average path length (0.08, 0.458, 0.856 and 1.121) than FP-G (0.046, 0.2, 0.565 and 0.516). Moreover, taxa were grouped in 4 co-occurrence modules and 29 taxa (Table S10) were identified as main module hub nodes (nodes highlighted in red) in FP-C's network (Fig. 6a). For FP-G (Fig. 6b) taxa were grouped in 7 modules and 21 taxa (Table S11) were identified as main module hub nodes.

Table 1 Summary of the comparison of the co-association network properties of the two floodplain sites, the cropland floodplain (FP-C) and the grassland floodplain (FP-G)

Network properties	FP-C	FP-G
Relative LCC size	0.08	0.046
Clustering coefficient	0.677	0.886
Modularity	0.458	0.2
Positive edge percentage	99.061	100
Edge density	0.161	0.467
Natural connectivity	0.22	0.537
Vertex connectivity	1	1
Edge connectivity	1	1
Average dissimilarity	0.856	0.565
Average path length (APL)	1.121	0.516

Discussion

While the prokaryotic community composition and functional differences of croplands and grasslands have been widely examined (Madegwa and Uchida 2021; Yang et al. 2022), the aim of this study was to determine how agricultural management directly or indirectly affects the soil bacterial and archaeal communities of an adjacent downslope floodplain site. We investigated two catenas which contained a floodplain site downslope of the agricultural sites, which included a cropland and a grassland. At all sites bacterial abundance was highest in summer, which is in agreement with prior studies that showed higher temperatures, nutrient availability and plant productivity increase prokaryotic abundance during summer months (Bardgett et al. 1999; Griffiths et al. 2003; Sarathchandra et al. 1988). Archaea did not follow this abundance trend. However, these effects were more pronounced in the cropland catena, compared to the grassland catena. Whereas these results could be expected for the cropland itself (Abrahão et al. 2022; Schäfer et al. 2019), the stronger increase in bacterial abundance at FP-C compared to FP-G was unexpected. Given the similar organic fertilization applied to both cropland and grassland, it might be considered that tillage, which was solely applied to the cropland site, might have played a role to explain this observation. Although tillage typically reduces soil organic carbon in agricultural topsoil (Haddaway et al. 2017), it may enhance nutrient availability in downslope areas through increased leaching (Jackson et al. 2003; Xiao et al. 2019), which at the same time can stimulate prokaryotic biomass growth (Carrero-Colón et al. 2006). This effect could be particularly significant in sloped areas, where subsurface leaching and surface runoff might be more pronounced (Wang et al. 2019b).

As no differences in TC and TN between the two floodplain sites were detected in our study, the observed bacterial abundance differences between the two floodplain sites might have been solely caused by the downslope transport of microorganisms. Obviously, this impact is highest at times of most intensive agricultural management, as bacterial biomass increased in FP-C as well as the shared ASVs between the cropland and FP-C, which was highest from March to June. Another factor that could have influenced our results was the relatively wet late spring or early summer in 2022 (Fig. S6), which might have increased downslope transport specially in June, while after a short drought period in summer (Fig. S6), transport became less important later in the year. This process might be especially relevant in case of flooding events along the year, following the translocation model that Collender et al. (2016) proposed. Although no flooding events were registered at the site in 2022 (Rebecca Hoess 2024, personal communication).

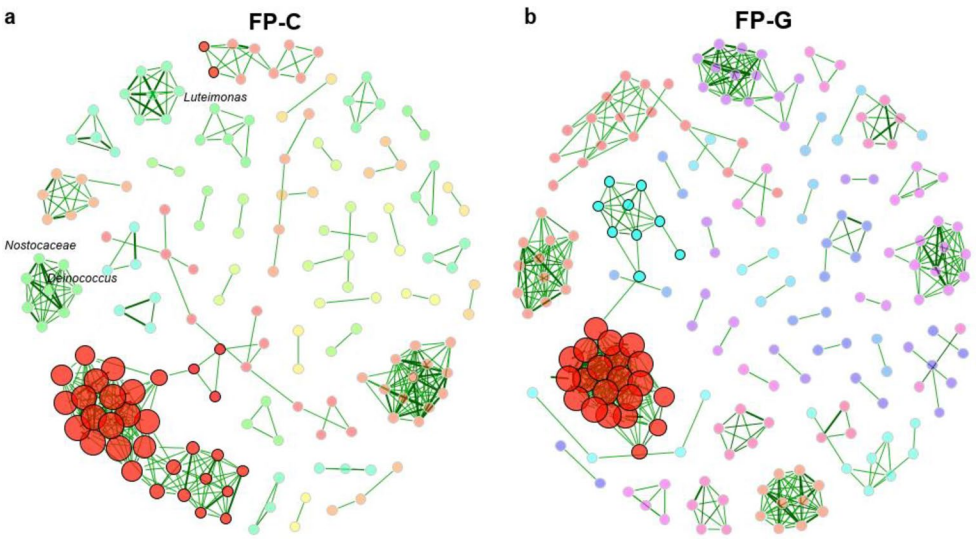

Fig. 6 Comparison of co-occurring prokaryotic taxa networks (nodes) at the ASV level based on the Spearman coefficient as co-association measure at the cropland floodplain (FP-C; **a**) and grassland floodplain (FP-G; **b**). Edges (connectors) in green represent positive associations and their width is proportional to the correlation. Colours are based on the cluster. Main module hub nodes are highlighted in red. Node sizes are based on eigenvector centrality

Crop management alters the prokaryotic composition of a downstream grassland floodplain

From the differential abundance analyses results and the prokaryotic networks, a fingerprint of the cropland management effect on FP-C could be identified. Regarding the former, several ASVs which were differentially abundant in the cropland compared to the grassland could also be detected as differentially abundant in FP-C. These ASVs were classified as *Nitrospira*, *Deinococcus*, *Nitrosophaeraceae* and the class *Nitrososphaeria.* Of those, *Nitrospira* is potentially able to carry out the complete nitrification process (Daims et al. 2015). *Nitrosophaeraceae* and the class *Nitrososphaeria* harbour known ammonia oxidising archaea (AOA), and their proliferation has been previously linked to fertilisation regimes in agricultural land (Megyes et al. 2021; Zhao et al. 2023). Although they were not identified as differentially abundant in the cropland, ASVs assigned to the acidophilic AOA *Nitrosotaleaceae* and *Nitrosomonadaceae* Group 1.1c, which could also be related to the fertilization-derived land use effect (Zhao et al. 2022; Zhalnina et al. 2012), were detected using this approach in FP-C.

Further proof of the effect of the cropland on the prokaryotic community composition of FP-C could be found when examining FP-C's prokaryotic network, as ASVs classified as *Luteimonas*, *Nostocaceae* and *Deinococcus*, which were highlighted as differentially abundant in the cropland compared to the grassland soil, were present in the network and were rather well connected at FP-C. *Luteimonas* has been previously described as a soil-borne plant pathogen (Zhang et al. 2017), while the family *Nostocaceae* consists of cyanobacteria that can perform aerobic N and C fixation, and initiate biological soil crust formation (Kurth et al. 2021), and *Deinococcus* is a well-known root-colonizing bacterium, which is also highly resistant to drought and low nutrients (Drigo et al. 2017; Santhanam et al. 2017).

Furthermore, the main module hubs of FP-C featured several plant-associated taxa, which were also found in the prokaryotic communities' core of the cropland and FP-C at the three sampling times, exemplified by *Streptomyces*, *Brevibacillus* and *Reyranella*, of which the first and second are known plant growth-promoting bacteria (PGPB) which are able to supress plant disease (Al-Quwaie 2024; Nehra et al. 2016). *Reyranella* has also been previously isolated from agricultural soils (Lee et al. 2017). Interestingly, despite there might have been a flow of single prokaryotic species from the cropland to FP-C, overall prokaryotic richness of FP-C was not affected and was comparable to FP-G throughout seasons. However, the mechanism of exchange is not clear from the data.

Regarding the effects on ecosystem services and biodiversity that the observed agriculture influence might have, the increase in abundance of nitrifying taxa can affect the cropland-influenced floodplain site in different ways. On the one hand, this shift might increase nitrates, which plants can readily absorb and use for growth, therefore enhancing soil fertility and plant productivity (Vidal et al. 2020). On the other hand, excess nitrate may lead to nutrient imbalances in plants and increase nitrate leaching into nearby water systems (Di and Cameron 2002). Which is particularly concerning in floodplains, in which proximity to stream ecosystems and groundwater heightens the risk of water contamination and potential eutrophication (Hallberg et al. 2022). Moreover, nitrifying bacteria contribute to the production of nitrous oxide (N_2O), a strong greenhouse gas, for whose emissions floodplain areas are already on the spotlight (Ley et al. 2018). It might be also worth to mention that other effects such as biodiversity loss were avoided with the use of organic fertilization at the site, as it can have a positive effect on the complexity of prokaryotic communities, as it could be seen in the prokaryotic networks comparison, and also on the keystone taxa (Luo et al. 2023).

Grassland management impacts on the bacterial and archaeal composition of a downstream grassland floodplain are low

The grassland extensive management did not have a significant influence on bacterial and archaeal biomass and prokaryotic richness in soil samples from FP-G, as biomass shifts followed seasonal trends and its observed ASVs number remained close to 1,500 ASVs at all the three sampling times. Contrarily to the influence of the cropland over FP-C described above, the differentially abundant ASVs of the grassland could not be detected as differentially abundant in FP-G and were also not found in the prokaryotic network. A reduced influence of grassland management on FP-G was expected, given that the grassland remained relatively undisturbed with no tillage and a stable plant cover over the season (Li et al. 2021; Wang et al. 2019a).

Agricultural management yearlong effect over the cropland floodplain and reset in winter

The progressive divergence of the bacterial and archaeal community composition of the sites over time, as indicated by the PCoAs clustering, went in parallel with the trend observed in the overall prokaryotic communities' core of the four sites, where numbers of common ASVs decreased progressively along the year, indicating a major external influence shifting their prokaryotic communities apart. Disturbances such as tillage, which can alter soil aggregation and structure (Liu et al. 2021; Schlüter et al. 2018) and a frequent vegetation cover variation might have made the

cropland more susceptible to seasonal changes (Li et al. 2021), while the grassland and floodplains remained relatively undisturbed and with a stable vegetation cover. Interestingly, the reduced management of all sites during winter may induce a reset in the bacterial and archaeal communities, even at the two floodplain sites. This is supported by our observation that prokaryotic community composition showed the least divergence in early spring, while the greatest differences were found in autumn. Additionally, Hu et al. (2023) emphasized that the most significant changes in prokaryotic community composition occur in spring, particularly among rare taxa, while the community tends to stabilize during the rest of the year. Thus, management-free of sites under agricultural use may play a crucial role in resetting bacterial and archaeal communities, not only at the directly managed sites but also at indirectly influenced locations, such as the floodplain sites in our study. Previous studies have also suggested the importance of this phase in restoring systems to their original equilibrium (Rinnan et al. 2009). Moreover, surface frost and ice during wintertime might also contribute to this reset by hindering downslope transport.

Conclusions

Our data clearly indicates significant implications of agricultural management on directly connected grassland-based floodplains in the composition of bacterial and archaeal communities as well as in the bacterial abundance. Although archaeal abundance and alpha diversity were not affected, the observed prokaryotic community shifts may have important consequences for soil health and ecosystem functions, particularly in the nitrogen cycling and productivity, as indicated by the effects on nitrifying prokaryotes. To fully understand these impacts, a functional approach would be required to determine if these findings are also mirrored on the functional side of the soil prokaryotic communities and subsequent activities.

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Author contributions The experimental design and funding acquisition was performed by Michael Schloter, Michael Dannenmann and Stefanie Schulz. Sampling was carried out by Rubén Martínez-Cuesta. Plant species identification was carried out by Anna Holmer. Nutrient measurements were performed by Franz Buegger. Samples processing, sequencing, bioinformatic processing and data analysis were carried out by Rubén Martínez-Cuesta. The first draft of the manuscript was written by Rubén Martínez-Cuesta, Michael Schloter and Stefanie Schulz. All authors have read and approved the final manuscript.

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Data availability Raw amplicon sequences can be found at the NCBI Sequence Read Archive (SRA) under the BioProject PRJNA1002989. The code used for the bioinformatic processing, statistical and ecological analyses of the amplicon sequencing data can be found at https://github.com/rubmc97/blik_1_exp.

Declarations

Conflict of interest The authors declare no conflict of interest.

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