

Article

Straw Management and Slurry Application Affect the Soil Microbial Community Composition and Its Activity

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Abstract: In low-input farming systems, where plant nutrition strongly depends on either organic matter inputs or nitrogen fixation by legumes, it is crucial to understand the effects of different crop residue management strategies on microbially mediated processes that influence nutrient dynamics. To evaluate different straw management options, we conducted a pot experiment with the following treatments: (1) control with no straw added (CON), (2) applying straw to the soil surface (SRF), simulating no-till, and (3) straw incorporating straw to the top 10 cm (INC), simulating minimum tillage. Another factor was slurry fertilization (with 3.2 t d.m. ha⁻¹ or without). Throughout the experiment, CO₂ efflux (μmol/m²/s) was measured regularly over a period of four months. Soil sampling was performed to monitor the abundance of the total bacterial, crenarchaeal and fungal communities, as well as nitrogen cycle microbial guilds and the dynamics of labile N and C fractions in the soils at different depths. Among straw treatments, significantly higher soil respiration was observed in INC and SRF compared to CON. Slurry fertilization increased soil respiration in all straw treatments. In the top 10 cm soil layer, the fungal abundance increased in INC compared to SRF and CON, whereas bacteria and crenarchaea did not respond. This effect was even more pronounced with slurry addition. The slurry also stimulated the abundance of certain N-cycle guilds, with the greatest increase in bacterial *amoA*-bearing nitrifiers and *nirS*, *nosZI* and *nozII*-bearing denitrifiers, particularly in the uppermost 0–1 cm soil layer. Straw treatment effects on N-cycle microbial guilds were less pronounced, while the coupled effect of straw incorporation and slurry was beneficial mostly for *nosZ*, again mainly in the top 1 cm soil layer. Compared to straw management, slurry fertilization had more potent effects on the abundance and performance of the investigated microbial communities.

Keywords: soil respiration; plant residuals; no-till; conservation tillage; ammonia oxidizers; denitrifiers; functional groups; nitrogen cycle



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1. Introduction

Leaving crop residues on croplands is an important, environmentally friendly approach to maintaining and increasing organic carbon contents and soil fertility [1,2]. Tillage practices determine the initial location and distribution of crop residues in the soil, either with crop residues remaining on the soil surface (such as in no-till systems) or with the incorporation of crop residues into the soil in conventional plow-tillage and reduced tillage systems. Affecting the residue–soil contact and vertical distribution of residues in the soil, soil tillage practices affect soil conditions, such as water content, soil aeration and temperature, and distribution of soil organic carbon (SOC) and plant-available N during the post-harvest period. Conservation tillage (no-till or reduced tillage) with organic residues left on the soil surface or incorporated into the 0–10 cm soil layer generally results in higher N and SOC content in the surface layer than conventional plow-tillage [3,4], which may affect residue decomposition processes and, in turn, the C- and N-cycles.

The soil microbial community plays an important role in straw decomposition and nutrient cycling in agroecosystems, largely affecting soil nutrient availability and dynamics by mobilization and immobilization processes. Vertical distribution of organic residue,

its chemical quality (C/N ratio, C/P ratio) and soil mineral N content [5–10] are among the major factors controlling the microbial decomposition rates of organic residues by modifying N access for microbial decomposers. Studies reported that incorporated residues have, in general, higher decomposition rates (measured by CO₂ efflux) than surface-applied crop residues [11,12], mainly due to the closer contact of the residues with the soil and thus more favorable soil conditions (soil water content, higher nutrient availability) for microbial decomposers. Decomposition rates also depend on the residue N content. For example, Abiven and Recous [13] reported differences in the decomposition of N-rich residues between straw left on the soil surface and straw incorporated into the soil. In contrast, residue management did not affect the decomposition of N-poor residues.

Changes in soil N dynamics induced by the straw input substantially affect soil microbial community dynamics [14]. An increase in microbial biomass in straw return systems was observed in many studies across a range of soil types and climates [15–17]. Moreover, conservation tillage and residue return influencing soil moisture and aeration creates conditions that could cause significant shifts in bacterial and fungal biomass ratios [10,18,19]. Due to the broader range of extracellular enzymes released by fungi, the soil microbial communities dominated by this group might have a higher capability to break down polymeric plant compounds (e.g., lignocellulose and cellulose) than those communities dominated by bacteria [20]. Studies reported that fungi and bacteria have an essential role in straw decomposition but at different phases of the process. Both fungi and bacteria utilize easily available carbon compounds in the initial phases of straw decomposition. In contrast, when labile C fractions are depleted in the later phases, fungi dominate, capable of using complex C sources [21–24].

Residues with a high C/N ratio do not meet the nitrogen needs of soil microbes. Combining residues with an additional N supply might affect the soil microbes and further enhance residue decomposition [25,26] and plant-available N for stubble crops. The application of organic or mineral fertilizers was reported to affect the composition, abundance, diversity, and functioning of soil microorganisms [25,27,28]. Several studies investigating the effect of mineral fertilizers combined with wheat straw or live-stock manures have shown that the application of organic fertilizers is more effective than the addition of straw in shaping soil microbial community composition and diversity, primarily due to favorable chemical quality (C/N, nutrient availability) and more balanced nutrient supply of organic fertilizers for soil microbes [29,30]. Furthermore, providing nutrients by adding organic matter (organic fertilizer, crop residues) is beneficial for the growth of microorganisms involved in the nitrogen cycle [31–34].

Certain agricultural practices (e.g., organic farming) strongly rely on organic matter inputs, such as manure and crop residue retention. Therefore, predicting the effects of such organic amendments on C and N transformation processes is essential in terms of potential nutrient and SOC losses and plant nutrient availability in the post-harvest period. Among others, the qPCR approach is commonly used to measure the abundance of certain functional microbial guilds involved in the nitrogen cycle. Nitrification and denitrification are the key processes in the nitrogen cycle that mediate N₂O emissions [35,36]. To assess the abundance of nitrifiers, the bacterial and archaeal *amoA* genes encoding ammonia monooxygenase, which catalyzes the microbial oxidation of NH₄⁺ to NH₂OH, are determined. Determination of the abundance of the *nirK/nirS* and *nosZI/nosZII* genes evaluates two steps in the denitrification process, namely the reduction of NO₂⁻ to NO, and of N₂O to N₂, respectively. The second phylogenetic clade of the *nosZ* sequence was identified [37], herein named *nosZII*. Studies have shown that *nosZII*-bearing denitrifiers play a greater role in the reduction of N₂O, compared to *nosZI*-bearing denitrifiers [38,39].

Previous studies investigating the effects of organic residue incorporation on N-cycling microbial communities in various tillage field experiments show shifts in the abundance of nitrifiers and denitrifiers in straw-amended soil [40–42]. However, the direct effect of residue placement in interaction with organic fertilization on the vertical distribution and composition of N-cycling microbial guilds is not yet known. Therefore, research

under more controlled conditions (e.g., pot experiments) is needed in parallel to observational field studies to better elucidate interaction effects between manuring and straw management options.

Thus, our main objectives were to investigate the effects of straw management (surface application vs. incorporated into the soil vs. control without straw) and slurry application (with and without), and their interactions on (1) available C and N dynamics in the soil at different soil depths, (2) microbial community composition determined by the abundances of total bacterial and Crenarchaeal 16S rRNA, and fungal ITS genes as well as genes of the key players of the nitrogen cycle, ammonia oxidizers (nitrifiers) and denitrifiers, and (3) activity of soil aerobic microbes measured by soil respiration.

2. Materials and Methods

2.1. Experimental Setup

The pot experiment was set up as two factors crossed design with time as additional (blocking) factor. Soil used in the pot experiment was Eutric Cambisol [43], developed on alluvial deposit. Soil material was collected from the unfertilized grassland site in Ljubljana, Slovenia. The main soil chemical properties are presented in Table 1. The soil was passed through 8 mm sieve and thoroughly mixed. Plastic cylinders ($d = 12.5$ cm, $h = 25$ cm) were filled with approximately 3 L of soil, up to a height of 1 cm below the edge of the pot. The experiment was carried out in the open field under a canopy. It lasted four months, from mid-August to mid-December, to mimic post-harvest stubble management in temperate areas of the northern Earth hemisphere. After two weeks of pot stabilization, wheat straw (5.2 t d.m. ha^{-1}) was either added to the soil surface (referred to as SRF) or homogeneously mixed into the 0–10 cm soil layer (referred to as INC) or was not added to the pots (CON). INC treatment corresponded to shallow straw incorporation frequently within the conservation tillage system. Straw was previously chopped to a 2–3 cm particle size. The chemical properties (C and N contents) of straw are shown in Table 1. An additional factor in the experiment was the application of cattle slurry (referred to as S), which was added two weeks after straw addition. Slurry application rate (3.2 t d.m. ha^{-1}) in this pot experiment was equivalent to 170 kg N ha^{-1} . There were thus six main treatments: (1) control—no plant residue and no slurry fertilization (CON), (2) straw applied at the soil surface (SRF), (3) straw incorporated in 0–10 cm soil layer (INC), (4) control with slurry fertilization (CON + S), (5) SRF with slurry fertilization (SRF + S) and (6) INC with slurry fertilization (INC + S), all carried out in four replications. Due to destructive soil sampling, four sets of pots per treatment were replicated five times, initially giving in total 6 treatments \times 4 replicated pots \times 5 temporal samplings = 120 pots. When slurry was added to SRF and INC pots, a corresponding volume of water was added to CON and CON + S pots to obtain the same water regimes for all treatments. Throughout the experiment, columns were regularly watered every 3–5 days to maintain water content equally for all pots. The amount of added water was based on soil water content measurements (Figure S1). We tried to maintain the soil water content in the range of 15 to 17%.

Table 1. Chemical properties of soil and wheat straw used in the experiment.

Chemical Properties	Soil	Wheat Straw
pH	6.7	
C _{total} (%)	1.7	
Carbonates (%)	1.2	
Organic matter (%)	2.7	
C _{org} (%)	1.6	45.1
N (%)	0.16	0.23

Table 1. *Cont.*

Chemical Properties	Soil	Wheat Straw
C/N	9.7	196.1
P ₂ O ₅ (mg/100 g soil)	<0.5	
K ₂ O (mg/100 g soil)	5.2	

2.2. Soil Respiration Measurements

To measure soil respiration, we randomly selected 24 pots (4 pots per treatment) at each time of respiration measurement. We measured soil CO₂ efflux ($\mu\text{mol m}^{-2} \text{s}^{-1}$) using LiCor 6400 portable photosynthesis measurement system (Li-Cor, Lincoln, NE, ZDA) equipped with a soil respiration chamber. A reduction cap was fitted between the pot and the Licor chamber to ensure air tightness. Soil respiration measurements were performed between 9:00 and 11:00, on days 1, 2, 3, 9, 10, 13, 17, 21, 28, 30, 36, 45, 59 and 77. At the beginning of the experiment, the measurements were more concentrated to catch the initial flush of microbial activity, thereafter, the frequency of measurements was decreased. The soil respiration was measured three times during a 3 min period for each pot per treatment, and averages of three replicates were calculated for each pot per treatment in every sampling period. In parallel with soil respiration measurements, we also measured soil temperature (Licor soil temperature sensor) and soil water content (SM-150; Delta-T Devices, UK) at 5 cm soil depth (Table S1).

2.3. Soil Sampling and Analyses

During the column experiment, soil samplings for destructive analysis were performed five times. Four replicate pots were destructively sampled on days 3, 13, 30, 49 and 84 (day one represented the day of slurry application). The soil in these pots was sliced into three layers: 0–1 cm, 1–10 cm and 10–20 cm, and the soil of each layer was divided into three parts. The first part was air-dried in the oven at 40 °C for 24 h, sieved through a 2 mm pore-size mesh sieve and used for chemical analysis (dissolved organic carbon (DOC) and inorganic nitrogen forms (NH₄-N, NO₃-N)), the second part was placed on dry ice and stored at –20 °C for DNA extraction and microbial analyses, and the third part was used for gravimetric soil water content determination. Based on the soil respiration measurements during the experiment, we choose samples from the 3rd, 13th and 49th sampling days to analyze the change in the soil microbial community. In addition, at the beginning of the experiment, before pots were filled with soil, the average soil sample was taken to determine baseline soil chemo-physical properties.

2.3.1. Soil Properties

For soil chemical analyses, samples were air-dried and sieved to 2 mm (ISO11464, 2006). Soil organic C (SOC) and total N (TN) were determined by dry combustion (ISO106941996; ISO138781987) using elemental analyzer (Elementar vario MAX instrument, Germany). DOC, nitrate nitrogen (NO₃-N) and ammonium nitrogen (NH₄-N) were extracted with 0.01 M CaCl₂ solution (ISO140551999) and determined using vario TOC cube (Elementar, Germany) for DOC, and Gallery Automated Photometric Analyzer (Thermo Scientific) for NO₃-N and NH₄-N detection. Carbonates were determined manometrically after soil reaction with HCl (ISO10693, 1995) and soil texture by the pipette method (ISO11277, 2009). Soil pH was measured in a 1/2.5 (*w/v*) ratio of soil and 0.01 M CaCl₂ suspension (ISO103902005). Soil water content was determined by oven drying at 105 °C to constant weight (ISO114651993).

2.3.2. Real-Time PCR (qPCR)

DNA was extracted from soil using the Power Soil DNA Isolation Kit (MoBIO Laboratories, USA) after a 5 min homogenization on Mini-Beadbeater-8 (BipSpec, USA), quantified

by NanoDrop 2000 UV–Vis spectrophotometer (NanoDrop 2000, Thermo Scientific, USA) and stored at -20°C until use.

Real-time PCR (qPCR) was used to quantify bacterial and crenarchaeal 16S rRNA [44,45] and fungal ITS genes [46]; the genes encoding catalytic enzymes of ammonia oxidation (nitrification) and denitrification. Nitrification potential in the soil was estimated by targeting bacterial (AOB) and crenarchaeal *amoA* (AOA) [47,48]. Denitrification potential was estimated using *nirK* [49] and *nirS* genes [50], and N_2O reduction potential was estimated using *nosZI* and *nosZII* genes [37,51]. All primers and PCR conditions are described in Table S1. Standard curves were obtained using serial dilutions of plasmid standards of respective functional genes ranging from 10^7 to 10^2 copies per reaction. Two technical replicates were used for each sample for each gene amplification. We use plasmid standards obtained from transformed *E. coli* cells (strain JM109) with inserted plasmid pGEM-T (Promega). All plasmid standards used for absolute quantification in this study were obtained from Dr. Laurent Philippot, Agroecology Department, INRA Dijon, France. The presence of PCR inhibitors in DNA samples was performed by diluting and spiking soil DNA with a known amount of an exogenous external control (the pGEM-T vector, Promega). In all cases, inhibition was not detected. Melting curves were analyzed for all runs to ensure PCR specificity.

Quantification was performed in a 384-well plate format using a QuantStudio™ 5 Real-Time PCR System (Applied Biosystems, CA, USA). Reactions were carried out in a 15 μL reaction volume containing 7.5 μL Absolute Blue QPCR SYBR Green Rox mix (Thermo Scientific), 1.5 μL of each primer (10 μM for AOA in AOB *amoA*, fungal ITS, bacterial and crenarchaeal 16S rRNA genes and 20 μM for *nirK*, *nirS*, *nosZI*, *nosZII* genes) and 2 μL of DNA template (1 ng/ μL). Additionally, we tested the addition of T4 gene 32 protein (500 ng/ μL) (MP Biomedicals, ZDA) to the reaction for each studied gene to improve the efficiency of gene quantification. According to the output, further T4 gene 32 protein was included in the reactions of crenarchaeal 16S rRNA, fungal ITS and *nirK* genes.

We obtained slightly different amplification efficiencies for different microbial genes as follows: 16S rRNA bacteria (89–93%), 16S rRNA crenarchaea (62%), fungal ITS (83–91%), *amoA* AOA (73–78%), *amoA* AOB (86%), *nirK* (90–93%), *nirS* (81–85%), *nosZI* (85–87%) and *nosZII* (79–80%) with R2 values of 0.997–1.000. Since we did not detect the presence of inhibitors in any of the soil-extracted DNA samples, we assume that the reason for the lower PCR efficiency, especially for crenarchaea, is more technical (e.g., primer design). We performed many tests to optimize protocol conditions and still could not achieve better efficiency.

2.4. Statistical Analysis

Soil chemical and microbial parameters were analyzed using a linear mixed model with treatment and sampling terms as between-units factors, pot as a random factor and soil-depth layer as within-units factor. For each parameter analyzed this overall model was evaluated graphically in terms of normality and variance homogeneity assumptions (histogram of residuals, q-q plot, box-plot). Additionally, more specific analyses of treatment effects were performed for each soil depth layer at each sampling term separately using two-way ANOVA, where the dependent variable was the measured parameter (e.g., gene abundances), and the independent variables were treatment combinations of straw management and slurry application (six levels) and sampling term. In this case, multiple comparisons of treatment levels were calculated using Tukey's test.

Soil respiration data were evaluated using a generalized additive model (GAM). The significance of straw management \times slurry application treatments was tested using a partial F-test by comparing the common GAM (joint model for all treatments) with the GAM with treatment factor included. To check the significance of either straw management options or slurry application, the subsets of data were produced, and separate GAMs were calculated on these subsets. Cumulative soil CO_2 emissions (in g C m^{-2}) were additionally calculated.

All tests were performed at $p < 0.05$ significance level. Data were analyzed in R environment [52] using packages nlme, multcomp and mgcv; for plotting ggplot2 package was used.

3. Results

3.1. Soil Respiration

Soil respiration, measured regularly throughout the experiment, showed a similar temporal pattern in all the treatments. A sharp increase occurred shortly after the addition of straw or slurry, followed by a sharp drop on day nine, a slight boost around day 21, and a slight decrease toward the end of the experiment that coincided with the autumnal drop in soil temperature (Figures 1 and S1). The largest effluxes were measured on days 2 to 3 in the treatments with slurry (INC and SRF), peaking at an average efflux of $15.5 \mu\text{mol m}^{-2} \text{s}^{-1}$ and $14.9 \mu\text{mol m}^{-2} \text{s}^{-1}$, respectively. Here, the highest variability was observed, decreasing with the lowering of the efflux magnitude (Figure 1).

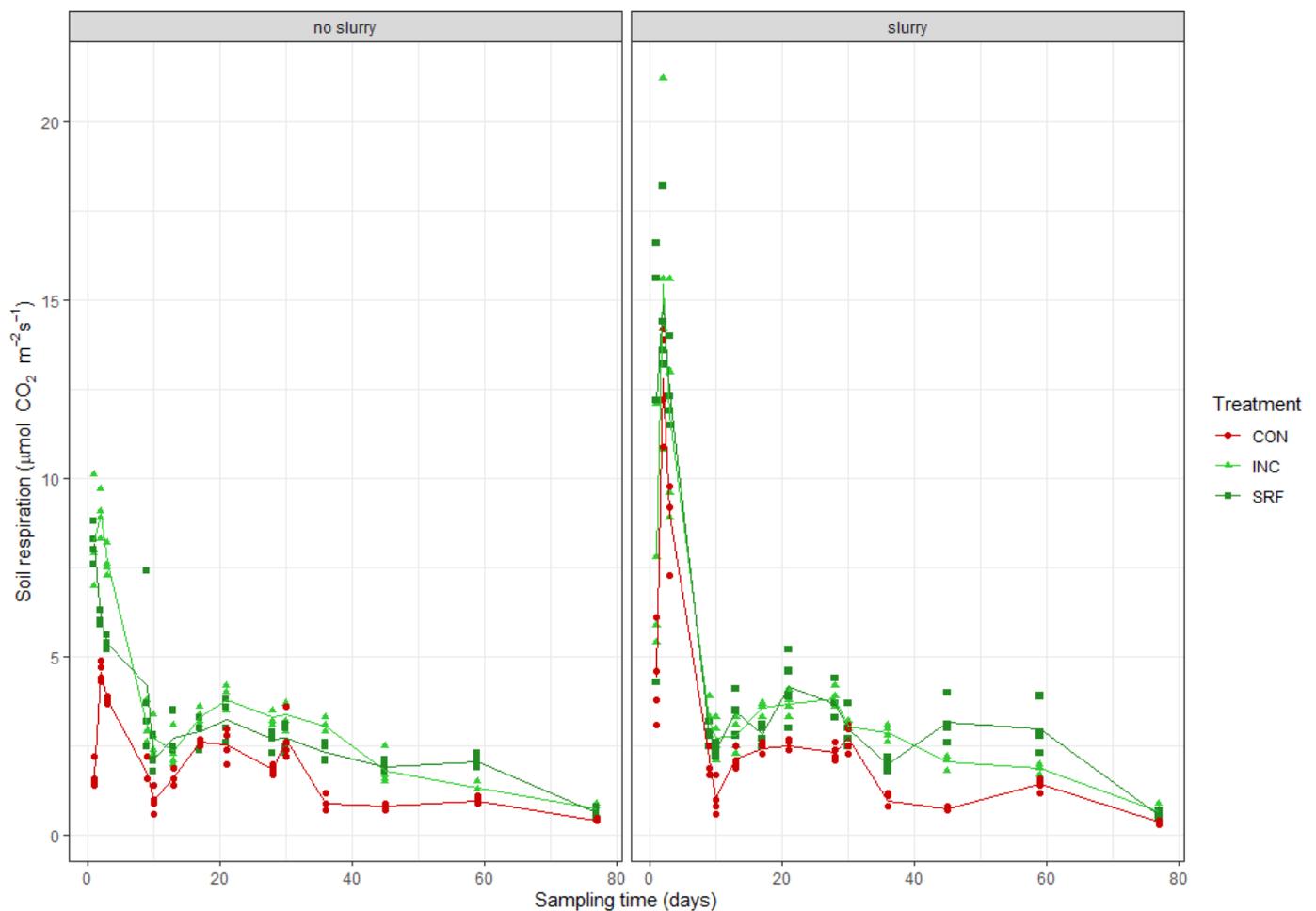


Figure 1. Dynamic of soil CO_2 effluxes ($\mu\text{mol CO}_2 \text{ m}^{-2} \text{ s}^{-1}$) in CON (control), INC (straw incorporated in 0–10 cm soil layer) and SRF (straw applied at the soil surface) soil treatments in presence/absence of slurry (S). Averages and raw data values of 4 replicates are shown.

Soil respiration was significantly affected by the addition of straw ($p < 0.001$) and slurry ($p < 0.001$) and their interaction ($p < 0.001$). Significant differences between straw management treatments were observed in the case when no slurry was added ($p < 0.001$), but not so for the pots with slurry fertilization ($p = 0.942$). Comparing unfertilized straw management treatments, significantly higher CO_2 efflux was observed in INC and SRF compared to the CON (without straw and slurry) ($p < 0.001$). Respiration rates in CON

remained the lowest throughout the experiment. Additionally, the calculated cumulative effluxes (g C m^{-2}) in INC and SRF were higher by 80.4% and 71.3%, respectively, compared to the CON treatment (Figure S3). Regardless of whether the straw was added or not, slurry (+S) significantly increased CO_2 emissions from soil (Figure 1), indicating a generally larger effect of slurry fertilization compared to straw management treatments. Larger stimulation of CO_2 efflux was observed for CON and SRF treatments upon adding the slurry, whereas the response for INC was minimal and confined to the initial phase of the experiment. For slurry treatments, INC + S, SRF + S and CON + S, the maximum average CO_2 effluxes were 72.3%, 144.6%, and 180.8% higher, respectively, than in unfertilized treatments. Thereafter, CO_2 effluxes decreased but were still higher in slurry fertilized treatments than in unfertilized treatments. At the end of the experiment (at day 77), the cumulative effluxes for all slurry-fertilized soil treatments were greater than for soil treatments without added slurry (Figure S3). On average, cumulative effluxes for INC + S, SRF + S and CON + S were 16.4%, 37.3% and 36% higher than for INC, SRF and CON treatments without added slurry, respectively. The highest cumulative efflux (274.5 g C m^{-2}) was observed in the SRF + S treatment.

3.2. Dissolved Organic Carbon and Nitrogen in Soil

We observed a decreasing trend in soil DOC with time in all treatments (Figure 2). With depth, DOC decreased; however, the difference in DOC content between depths was more pronounced in the treatments with slurry fertilization than in the unfertilized. Slurry fertilization had no impact on DOC content at 1–10 cm and 10–20 cm depths in SRF and CON treatments, while in INC, the lower DOC content was observed in the case of slurry fertilization.

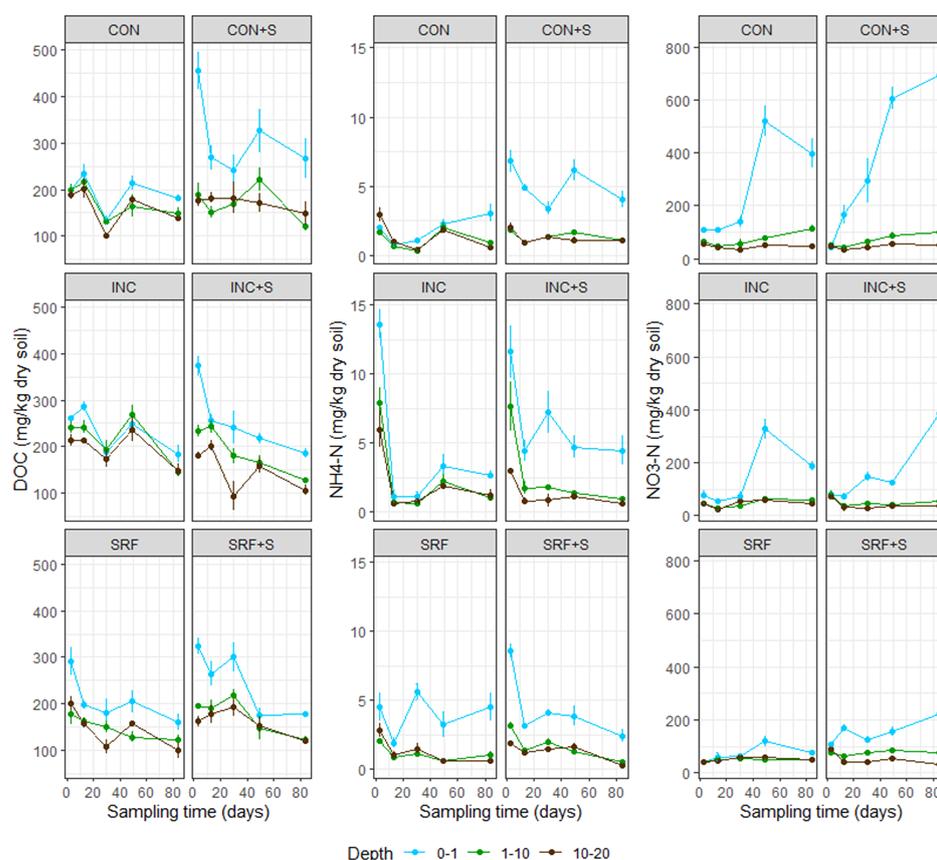


Figure 2. Dynamic of dissolved organic carbon (DOC) and mineral nitrogen forms ($\text{NH}_4\text{-N}$, $\text{NO}_3\text{-N}$) in CON (control), INC (straw incorporated in 0–10 cm soil layer) and SRF (straw applied at the soil surface) soil treatments in presence/absence of slurry (S) at three sampling depths (cm). Averages and standard errors of 4 replicates are shown.

In the initial phase of the experiment, soil NH_4^+ concentration was the highest in treatments with incorporated straw (INC and INC + S) at all sampling depths (Figure 2). Afterward, it first decreased continuously till day 13 and later remained almost constant. Slurry fertilization generally increased NH_4^+ concentration in the uppermost 1 cm of the soil, whereas lower soil layers were less affected. The exception is SRF, where slurry did not affect NH_4^+ concentration in the 0–1 cm soil layer.

Similarly to NH_4^+ , NO_3^- concentrations showed the largest response for the 0–1 cm soil layer. Here, NO_3^- increased over time in all treatments, both with and without slurry fertilization, except SRF. The highest NO_3^- increase was observed in CON with and without slurry. We also observed an increasing trend in NO_3^- concentration at 1–10 cm depth in CON with and without slurry, while concentrations in the other soil treatments at 1–10 cm and 10–20 cm depth remained almost constant throughout the experiment (Figure 2).

3.3. Abundance of the Total Microbial Community in Soil

The bacterial 16S rRNA and fungal ITS gene abundances (Table S3) were significantly affected by treatment ($p < 0.0001$), sampling time ($p < 0.0001$), depth ($p < 0.0001$) and their interaction ($p = 0.002$), while the abundance of crenarchaeal 16S rRNA genes was affected only by treatment ($p < 0.0001$), sampling time ($p < 0.0001$) and their interaction ($p < 0.0001$) (Table S2). In the top 10 cm of soil, INC significantly increased the abundance of fungal community, but not bacterial and crenarchaeal, compared to the CON and SRF. In the last sampling term, fungal abundance in the 1–10 cm soil layer of INC was on average 140% higher than in CON. This effect was even more pronounced in treatments with slurry fertilization. Slurry significantly increased bacterial and fungal abundance in the 0–1 cm and 1–10 cm soil layers in INC and CON, but not in SRF. The depth effect on bacterial and fungal abundance was also greater in treatments with slurry fertilization, while the crenarchaeal abundance was rather evenly distributed with depth (Figure 3). The abundance of crenarchaea was increased by slurry only in the CON treatment at all three sampling depths. In the INC treatment, fungi were more responsive to the straw addition and slurry fertilization than bacteria (Figure 3). When comparing straw management treatments, the highest average fungal/bacterial ratios were observed in the top 10 cm soil layer in INC and SRF, both fertilized and unfertilized, compared to the CON (Table S8). Observing the entire sampling period, we noted a decrease in the abundance of fungi and bacteria in CON and INC on the 13th day of the experiment (2nd sampling point), but not in the slurry treatment. The same pattern was observed for *amoA* AOA nitrifiers, as well as all denitrifiers, and could be related to the decreased ammonium content due to mineralization in the straw-amended soil, while the slurry increased mineral nitrogen in the soil, which could have a stimulatory effect on soil microbes.

3.4. Abundance of N-Cycling Microbial Communities in Soil

3.4.1. Abundance of Nitrifiers

The abundance of bacterial *amoA* (AOB) and archaeal *amoA* (AOA) genes in soil (Table S4) were significantly affected mostly by depth and sampling time but less by treatment (Table S2). The depth response was more pronounced for AOB than for AOA, especially in the treatments with slurry fertilization (Figure 4). The slurry significantly increased AOA and AOB abundances ($p < 0.0001$), but this effect varied across the straw addition treatments. The differences in the abundance of AOA and AOB between treatments with and without slurry fertilization appeared at the second sampling time (day 13). AOA increased in all three depths in the INC and CON treatments but not in SRF. The abundance of AOB significantly increased in 0–1 cm and 1–10 cm soil layers in all treatments, with the greatest increase in the top 0–1 cm soil layer (Figure 4). When comparing sampling times, a decrease in AOA abundance was observed in CON and INC at all sampling depths on the 13th day of the experiment (2nd sampling point), while in SRF the abundance of these genes increased in the soil layers of 1–10 and 10–20 cm. In contrast, the abundance of AOB remained unaffected by straw addition. We also found that AOA *amoA* genes were more

abundant than AOB. On average, the ratio of AOA to AOB ranged from 0.8 to 27.2, with the lowest ratios in the top 10 cm of slurry-fertilized soils (Table S7).

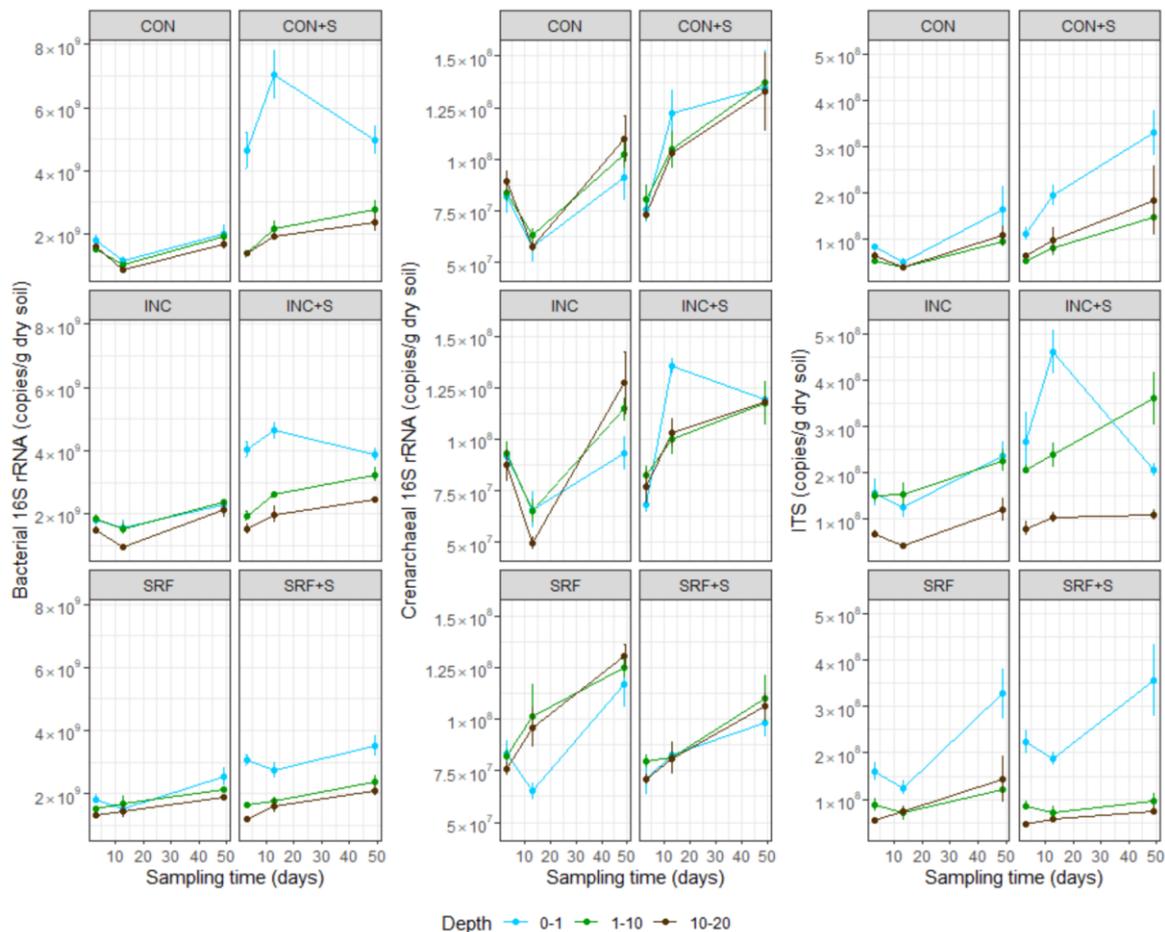


Figure 3. Dynamic of bacterial, crenarchaeal 16S rRNA and fungal ITS copy numbers in CON (control), INC (straw incorporated in 0–10 cm soil layer) and SRF (straw applied at the soil surface) soil treatments in presence/absence of slurry (S) at three sampling depths (cm). Averages and standard errors of 4 replicates are shown.

3.4.2. Abundance of Denitrifiers

The abundance of denitrification *nirK*, *nirS*, *nosZI* and *nosZII* genes in soil was significantly affected by treatment, sampling time and depth (Table S2). On the 13th day of the experiment, in all treatments with straw addition and without slurry fertilization, we observed a decrease in the abundance of all denitrification genes at all depths. Comparing straw treatments, we observed a higher abundance of *nirK* genes, especially in the upper 1 cm of SRF and INC compared to CON. However, the differences were still not significant throughout the whole sampling period. The slurry fertilization significantly increased the abundance of *nirK* and *nirS* genes in the top 1 cm of soil in CON and INC but not in SRF. In the deeper soil layers, the effect of the slurry was less pronounced or even not significant (Figure 5). In the 1–10 cm soil layer, slurry fertilization significantly increased the *nirS* gene abundance only in CON at the second sampling time and in INC and CON at the last sampling time, whereas in the 10–20 cm soil layer, slurry fertilization increased *nirS* only in INC and CON at the second sampling time. We also found that the *nirK* gene was more abundant than *nirS* gene. On average, the ratio of *nirK* to *nirS* ranged from 3.6 to 13.4, with the lowest ratio in the slurry-fertilized soils (Table S7).

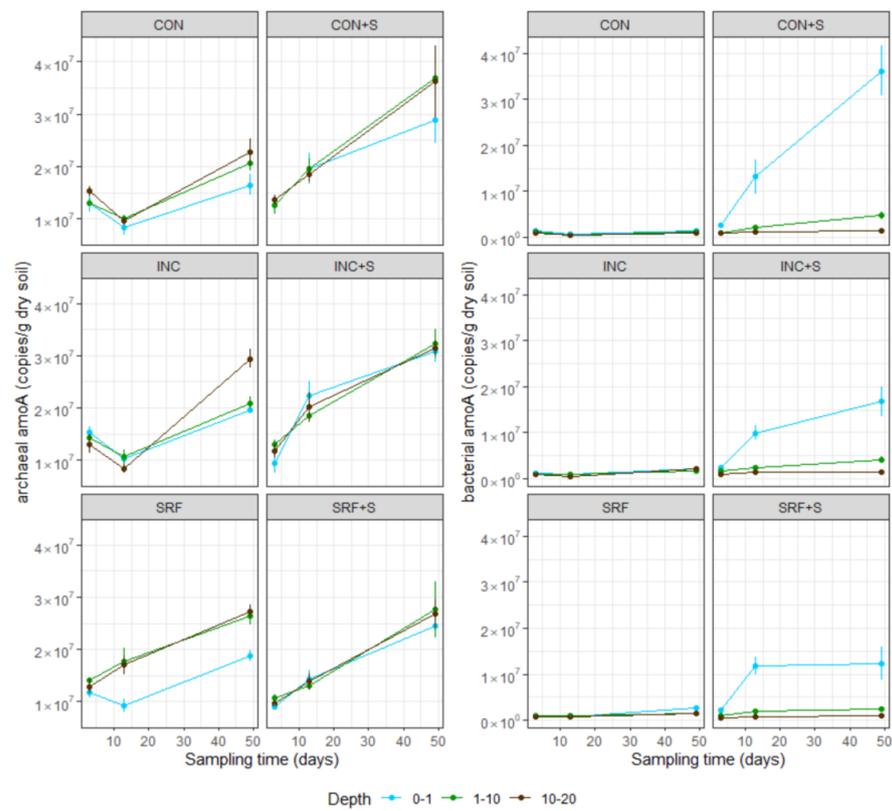


Figure 4. Dynamic of crenarchaeal (AOA) and bacterial (AOB) *amoA* gene abundance in CON (control), INC (straw incorporated in 0–10 cm soil layer) and SRF (straw applied at the soil surface) soil treatments in presence/absence of slurry (S) at three sampling depths (cm). Averages and standard errors of 4 replicates are shown.

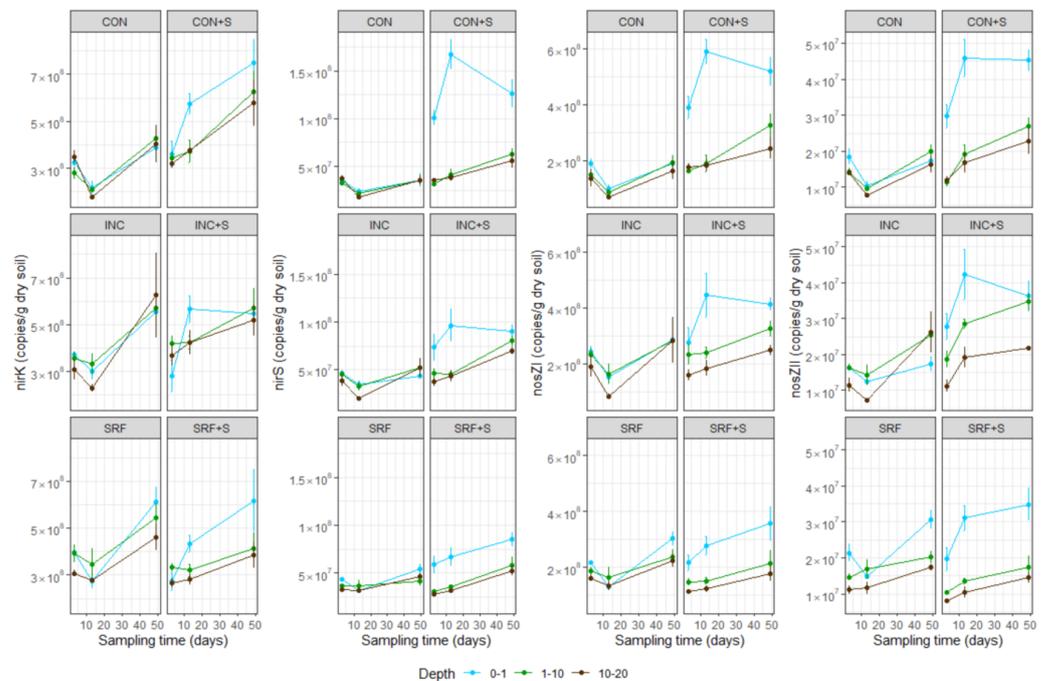


Figure 5. Dynamic of *nirK*, *nirS*, *nosZI* and *nosZII* gene abundance in CON (control), INC (straw incorporated in 0–10 cm soil layer) and SRF (straw applied at the soil surface) soil treatments in presence/absence of slurry (S) at three sampling depths (cm). Averages and standard errors of 4 replicates are shown.

The abundance of *nosZI* and *nosZII* genes were not affected by straw addition, while slurry fertilization increased the abundance of *nosZI* gene in CON soil at all depths and in INC soil only in the 1 cm. Slurry fertilization also increased the abundance of the *nosZII* gene in the 0–1 cm and 1–10 cm in INC treatment compared to other treatments. Differences in *nosZI* and *nosZII* abundances between depths were more pronounced in slurry-fertilized than in the unfertilized treatments (Figure 5; for ratios between gene abundances also see Table S9). The *nosZI/nosZII* ratio did not change with slurry fertilization, while it was increased in the 0–1 cm of INC compared to SRF and CON treatment (Table S7).

4. Discussion

Here, a pot experiment was conducted to observe the patterns of soil microbial activity and functional composition influenced by two specific agricultural practices associated with cereal stubble management, namely crop residue management and manuring. Both are important for soil fertility, water retention, soil erosion control, and the growth and yield of subsequent crops. In this context, it is necessary to understand the underlying soil processes that are affected by these practices, especially those mediated by soil microbes. Field trials are the preferable option to achieve this but the interplay of many factors interacting in time and space in the field (weather effects, soil variability, uneven tillage or fertilization, etc.) often makes it difficult to discern the effects of the investigated treatments. This is especially the case when small differences in measured parameters are expected. Therefore, despite their limitations [53], pot experiments are still considered a valuable tool to thoroughly evaluate the effects of individual experimental factors and their interactions. The microbial mechanisms are thought to be the same regardless of the experimental type, yet the magnitudes of the measured response variables (e.g., soil respiration, DNA copy number, DOC/DON concentrations in our case) are difficult to extrapolate to the field level.

4.1. Effects of Straw Management and Slurry Fertilization on Soil Respiration

Our data show that straw distribution (either distributed into 0–10 cm of soil or applied to the soil surface) and slurry fertilization significantly affected the magnitude and dynamics of emitted CO₂ (Figure 1). It is important to interpret the soil respiration results in terms of the possible C sources of each straw and slurry treatment. Since we did not include a plant component in our experiment, the observed soil respiration reflects microbial (heterotrophic) respiration. However, the source of emitted CO₂ cannot only be attributed to the mineralization of straw or slurry but also to the mineralization of native soil organic matter (nSOC) [54,55]. In addition, physical soil disruption during straw incorporation could promote the mineralization of nSOC reserves [56,57]. Therefore, the observed differences between CON and INC may be attributed to straw mineralization and this additional mineralization of nSOC. The priming effect (accelerated nSOC mineralization after the addition of a labile C source) [58,59] cannot be excluded when comparing the soil respiration results of the slurry and straw treatments with those of the control treatments (Figure 1).

Our results are consistent with the observations of many studies, observing increased soil CO₂ emission rates after adding organic residues to soil [21,60,61]. When soils are C-limited, adding labile C and N from organic residues facilitates the growth of copiotrophic organisms in the early phase of straw decomposition [21,61,62]. Consistent with these findings, in the straw-amended soil treatments, we observed a sharp increase in microbial respiration during the first three days during microbial colonization of fresh organic matter and a gradual decrease afterward (Figure 1) when easily decomposable organic compounds were consumed. When supplying C-rich and nutrient-poor crop residues (C/N ratio higher than ~20), soil nutrient availability is a limiting factor for the biotic decomposition of residues, especially in the early phases [63]. In this study, and in agricultural practices as well, the slurry was added to the soil to mitigate a possible N limitation during residue decomposition. In the initial phase (0–3 days), the slurry fertilization increased CO₂ emissions in all treatments, regardless of if the straw was added or not or how it was distributed

(Figure 1). This is consistent with many other studies that observed an increase in respiration rate when organic C amendments were combined with exogenous N supply [54,59,60]. Slurry fertilization positively affected the release of dissolved organic carbon during the decomposition of straw, especially in the top 1 cm of soil (Figure 2). This may be attributed to the improved environmental conditions caused by slurry fertilization, which is consistent with the findings of Liu et al. [63], who studied wheat straw decomposition dynamics in a field under different nitrogen fertilization regimes. In our study, the slurry fertilization increased available N contents (Figure 2), promoted microbial activity, and thus accelerated microbial respiration. The decreasing trend of DOC content in all treatments of our study (Figure 2) seems to be related to the observed respiration trend (Figure 1) [21]. However, from the experimental design, we cannot discern whether the slurry was beneficial because of its labile N content, its labile C content, or both.

For slurry-amended pots, we did not observe significant differences between our straw management treatments. These results differ from other studies, where lower CO₂ emissions from residue non-amended soils with N addition were reported, compared to residue-amended soils with N addition [59,64]. For example, the authors of [59] observed a negative priming effect when mineral N was added alone, whereas the addition of organic C together with mineral N accelerated the mineralization of nSOC, resulting in a positive priming effect. Since we used grassland soils in our experiment, it is possible that some plant material still remained in the soils despite sieving, and the slurry fertilization in control (straw non-amended soils) accelerated the decomposition of this remaining plant material and nSOC in the soil, resulting in increased CO₂ emissions [60].

We showed that the differences in microbial respiration between INC and SRF treatments occurred mainly during the initial phase. In INC treatment, where the straw was homogeneously distributed in the soil, the microbial respiration in the initial phase was greater than in SRF, where the contact between straw and soil was poor. This is in accordance with several studies showing that closer straw–soil contact provides more favorable abiotic conditions for microbial colonization and decomposition of plant residues [65–67]. Henriksen and Breland [67] showed that under poor residue–soil contact conditions, the growth of microbes capable of producing degradable enzymes is limited, which in turn leads to reduced decomposition of straw.

Furthermore, higher microbial respiration in INC than in SRF and CON may be influenced by the physical disruption of soil that alter the microenvironment and stimulate microbial activity [56]. During the initial phase (0–3 days) of the experiment, a higher ammonium content was observed in INC soils compared to SRF and CON soils (Figure 2). Given the high C/N ratio of the straw, this ammonium increase cannot be attributable to straw mineralization but is more likely to be the aforementioned nSOC mineralization, enhanced during soil disturbance. In addition, higher DOC content was found in soils amended with straw compared to the control without straw. After the initial peak of microbial respiration, differences between INC and SRF treatments were small (Figure 1), coinciding with a relatively constant trend of ammonium content in the soil (Figure 2). Similarly, the authors of [68] showed a significant effect of wheat straw position on its decomposition shortly after straw amendment; two weeks later, the effect of straw location on decomposition was weak, probably because the availability of inorganic N was limited.

The differences in microbial respiration between INC and SRF may also be partly related to soil moisture conditions and temperature and oxygen availability caused by physical disturbance of the soil in INC treatments [11,69,70]. In the present study, moisture and temperature were monitored in parallel with respiration measurements, and water content was maintained by regular, uniform watering of all pots. We observed a higher average moisture content in the SRF soil than in INC (Figure S2), indicating lower surface transpiration due to the shading effect of plant residues. The authors of [11] showed that higher moisture content under plant residues contributes to the increased microbial activity in the upper soil layers, stimulating the mineralization of organic matter. These observations are in contrast to our results, as we found higher microbial respiration in INC

soils, where soil moisture content was lower compared to SRF soils. Evidently, other factors, such as soil–residue contact and aeration conditions, had a greater impact on microbial respiration in our study.

4.2. Effects of Straw Management and Slurry Fertilization on the Total Microbial Community

It is well known that different residue management options influenced by tillage regimes alter soil physical and chemical properties, which may, in turn, affect soil microbial communities and their functioning [71,72]. In the present study, available C and N contents were increased in the straw-enriched treatments (INC and SRF) compared with CON (Figure 2). It is important to note that the straw applied to the soil surface (SRF) was not visibly degraded till the end of the experiment. Accordingly, the increase in DOC and nutrients in SRF soils was observed only in the top 1 cm layer. In contrast, the change in the INC soils was observed at all sampling depths during the initial phase of the experiment (Figure 2). These results suggest that with the enhanced decomposition, there was a greater release of many nutrients and DOC from the straw-enriched soils, especially in INC treatment, which may have stimulated microbial growth [15]. The abundance of fungi can confirm this hypothesis; fungi were significantly increased in the 1–10 cm soil layer of INC compared to SRF and CON soils (Figure 3). In contrast, bacteria and archaea did not respond to the addition of straw. These results are consistent with studies that showed rapid fungal growth by adding plant residues [22,73], particularly when comparing fungi with bacteria and archaea. The increased fungal/bacterial ratio in the 1–10 cm soil layer of INC was due to increased fungal abundance (Figure 3). In general, fungi were shown to have a strong competitive advantage in the utilization of straw over bacteria in normal cropland soil conditions. Fungi tend to dominate in the degradation of cellulose-rich litter compounds compared to bacteria which dominate in the degradation of the soluble fraction [24,32,73,74], especially at the earliest phases after straw addition [29]. Our results are consistent with the findings of Miller et al. [75], who indicated that the C additions used in their experiment (glucose, red clover, and barley straw) were not sufficient to cause a measurable increase in the total bacterial community. However, a greater bacterial contribution to residue decomposition may be possible with longer incubation periods [26].

Slurry fertilization increased the total bacteria, archaea, and fungi in the straw treatments by increasing available C and N (Figures 2 and S2). After slurry fertilization, bacterial and fungal abundances increased significantly in the top 0–10 cm of CON and INC treatments. In SRF, bacterial abundance was increased only in the top 1 cm, while fungal abundance remained unchanged after fertilization. The negligible effect of slurry in SRF can be explained by the fact that slurry was intercepted by the straw lying on the soil surface and consequently penetrated less into the soil. Interestingly, the fungal/bacterial ratio remained unaltered by slurry fertilization in all treatments, indicating that slurry does not preferentially promote any particular microbial community. The higher bacterial and fungal abundance resulting from slurry fertilization was accompanied by higher respiration rates (Figure 1), indicating a greater decomposition of straw.

In contrast to bacteria and fungi, an increased abundance of archaea due to slurry fertilization was observed only in CON soil at all sampling depths, while their abundance was not changed in straw-enriched soils without a slurry. These results indicate a fast response of the archaeal community to the increased availability of labile N forms in the soil due to slurry fertilization. Our results are consistent with studies in which increased archaeal abundance was observed due to organic fertilization [76] or mineral N fertilization without added wheat straw [77]. In contrast, the authors of [78] reported a lower abundance of Crenarcheota in soil with higher ammonium concentrations; however, the average ammonium concentration in this study (40.8 mg kg^{-1} of soil) was more than three times higher compared to our experiment. Generally, the effects of organic fertilizers on archaea show inconsistency across studies, and further work is needed to understand their response to different fertilization and soil tillage regimes.

4.3. Effects of Straw Management and Slurry Fertilization on the Nitrifiers and Denitrifiers

Microorganisms of different functional groups play a key role in the ecosystem's nitrogen cycle. Previous studies have shown that organic amendments increase the abundance of nitrifying [79,80] and denitrifying communities [33,81]; however, the combined effects of the organic amendment (slurry or straw) and organic residue distribution on microbes involved in the N-cycle have not been investigated yet. Our results support the findings of several other studies that variations in environmental conditions lead to niche differentiation among communities responsible for the same soil N transformation process [82–85]. In general, niche differentiation was observed in our study due to (i) soil disturbance, e.g., for *amoA*- and *nosZ*-bearing nitrifiers and denitrifiers, respectively; (ii) slurry fertilization, e.g., for *amoA*- and *nirS/K*-bearing nitrifiers and denitrifiers, respectively; (iii) soil disturbance and slurry fertilization between the two *nosZ* clades (*nosZI* and *nosZII*) (Table S7).

More specifically, our results suggest that AOA and *nosZII* communities are less sensitive than AOB and *nosZI*, respectively, to soil disruption due to mixing straw into the soil. Other authors obtained similar results for nitrifiers in long-term field tillage experiments [85,86]. Additionally, for denitrifiers, our results are consistent with the previous observation that the *nosZI* community responds more rapidly to C amendments than *nosZII* [84]. Considering the short duration of our study, it is also possible that adaptations of *nosZII* denitrifiers require longer periods. Interestingly, when observing temporal dynamics of denitrifier abundance among straw management treatments, *nirK*-bearing microbes preferentially were influenced by surface straw placement, significantly in the top 1 cm of soil (Figure 5; Table S5). Probably the higher water content in the upper soil layer stimulated their growth. These results are consistent with Szukics et al. [87], who reported that *nirK* gene abundance increased rapidly in response to wet conditions until NO_3^- became limiting. Several other studies also suggest that the *nirK* population inhabits different niches compared to the *nirS* population [87–89].

Slurry fertilization, in general, shifted the composition of *amoA*-bearing nitrifiers to AOB, and *nir*-bearing denitrifiers to *nirS* in the upper 10 cm of soil, which was observed as lower AOA/AOB and *nirK/nirS* ratios (Table S7). Our results are in line with previous observations showing ammonium stimulatory effect on AOB, whether added as ammonium solely [79,90,91] or manure [79,92,93]. In contrast to AOB, AOA prefers a low ammonium substrate environment for growth. Our results from the top 1 cm of soil also suggest that *nirS*-bearing denitrifiers are more sensitive to nitrogen addition (slurry) than *nirK*, as was previously obtained by other authors [94,95]. The combined effect of straw incorporation and slurry fertilization was beneficial for *nosZ*-bearing microbes in the top 1 cm soil layer. The higher water content in slurry treatments probably induced more anaerobic conditions, which is favorable for denitrifiers [75].

Finally, when interpreting our results on the composition of the soil microbiome, it should be emphasized that genetic analyses performed at the DNA level only represent the potential of specific processes [37,96], so DNA-based microbial abundance does not necessarily reflect microbial activity in the process. Nevertheless, this method is commonly used to assess the abundance of microbial populations involved in specific nitrogen-cycling processes [96,97]. However, biases related to DNA extraction from soil or low specificity of primers may occur, resulting in lower efficiency for some markers, such as Crenarchaea in our case.

5. Conclusions

As our results show, straw management and slurry fertilization, both important agricultural measures, had a significant impact on many soil processes, directly by adding C-compounds and nutrients, or indirectly by disturbing the soil and altering the environment. We found that straw incorporation enhanced the decomposition process more than surface-applied straw and that external nitrogen in the form of slurry enhanced decomposition while no significant effect of straw and slurry interaction was observed. However, from the design of our experiment, we cannot discern if the slurry was beneficial due to its

labile N contents, its labile C content, or both. The changes in soil respiration due to straw and slurry were reflected in the dynamics of the soil microbial community. Our results suggest that the combined treatments of straw incorporation and slurry fertilization are especially beneficial for the fungal community, triggering a more active nitrogen cycle than treatment without crop residues. Additionally, nitrogen availability and water content play an important role in regulating the nitrogen cycle. Our results highlight the niche differentiation among nitrogen-cycling genes involved in the same transformation process. However, we must remember that we conducted the study over a short period and that experiments over a more extended period might provide better insight into microbially mediated carbon and nitrogen cycling. Future studies should also include RNA-based approaches to quantify N-cycling guilds in parallel with N₂O emission measurements to better understand the underlying mechanisms.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/agronomy12112781/s1>, Table S1: qPCR primers used for quantification and temperature profiles; Table S2: *p*-values for the main effects of the different treatments, sampling time and depth, and their interactions for real-time PCR gene copy estimation (bacterial and Crenarchaeal 16S rRNA, fungal ITS, archaeal (AOA) and bacterial *amoA* (AOB), *nirK* and *nirS*, *nosZI* and *nosZII*) as determined by three-way ANOVA; Table S3: Bacterial, Crenarchaeal 16S rRNA and fungal ITS copy numbers in CON (control), INC (straw incorporated in 0–10 cm soil layer) and SRF (straw applied at the soil surface) soil treatments in presence/absence of slurry (S) at three sampling depths (cm) and three sampling points. Averages and standard errors of four replicates are shown; Table S4: Crenarchaeal (AOA) and bacterial (AOB) *amoA* gene abundances in CON, INC and SRF soil treatments in presence/absence of slurry (S) at three sampling depths (cm) and three sampling points. Averages and standard errors of four replicates are shown; Table S5: *nirK* and *nirS* gene abundances in CON, INC and SRF soil treatments in presence/absence of slurry (S) at three sampling depths (cm) and three sampling points. Averages and standard errors of four replicates are shown; Table S6: *nosZI* and *nosZII* gene abundances in CON, INC and SRF soil treatments in presence/absence of slurry (S) at three sampling depths (cm) and three sampling points. Averages and standard errors of four replicates are shown; Table S7: *amoA* AOA/AOB, *nirK/nirS* and *nosZI/nosZII* gene abundance ratios in CON, INC and SRF soil treatments in presence/absence of slurry (S) at three sampling depths (cm) and three sampling points. Averages and standard errors of four replicates are shown; Table S8: ITS/16S rRNA bacteria, *nirK*/16S rRNA bacteria and *nirS*/16S rRNA bacteria gene abundance ratios in CON, INC and SRF soil treatments in presence/absence of slurry (S) at three sampling depths (cm) and three sampling points. Averages and standard errors of four replicates are shown; Table S9: (*nosZI* + *nosZII*)/16S rRNA bacteria and (*nirK* + *nirS* + *nosZI* + *nosZII*)/16S rRNA bacteria gene abundance ratios in CON, INC and SRF soil treatments in presence/absence of slurry (S) at three sampling depths (cm) and three sampling points. Averages and standard errors of four replicates are shown; Figure S1: Measurements of soil water content (SWC) and soil temperature (T) at depth 10 cm in CON, INC and SRF soil treatments in presence/absence of slurry (S). Averages of four replicates are shown; Figure S2: Measurements of soil water content (SWC) in CON, INC and SRF soil treatments in presence/absence of slurry (S) at three sampling depths (cm). Averages and standard errors of four replicates are shown; Figure S3: Cumulative CO₂ effluxes (g C m⁻²) in CON, INC and SRF soil treatments in presence/absence of slurry (S). Averages of four replicates are shown.

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