Spring barley performance benefits from simultaneous shallow straw incorporation and top dressing as revealed by rhizotrons with resealable sampling ports

Rüdiger Reichel1 · Catherine Wambui Kamau2 · Amit Kumar3 · Zhijie Li1 · Viviane Radl2 · Vicky M Temperton3 · Michael Schloter2 · Nicolas Brüggemann1

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Abstract
Plant residues with larger carbon (C) to nitrogen (N) ratios can stimulate microbial growth and thereby protect soil nutrients from leaching. In poorly fertilized soil, excessive immobilization may limit nutrient availability and thus plant growth. Little is known about the impact of a shallow straw incorporation on soil microbial regulation of top-dressing fertilizer nutrients and spring crop establishment. We aimed to evaluate if wheat straw in combination with mineral fertilizer has more positive effects on plant performance than mineral fertilization alone and if this relates to changes of the extractable C:N:P ratio and microbial activity close to the roots. In order to conduct small-scale sampling with minimal disturbance during growth of spring barley (Hordeum vulgare L.), we developed rhizotrons with resealable ports. Rhizotrons were filled with loamy-sandy soil and fertilized with an equivalent of 150 kg N and 80 kg P ha−1. Half of the rhizotrons received the top dressing together with 4500 kg wheat straw-C ha−1. Throughout a 90-day greenhouse experiment, we analyzed soil C:N:P dynamics, and carbon dioxide (CO2) and nitrous oxide (N2O) emission, together with microbial biomass, selected bacterial genes (abundance), and transcripts (activity) in bulk and root-affected soil at multiple times. We focused on nitrifiers and denitrifiers and linked our data to barley growth. Interactions between straw and roots caused shifts towards larger C:P and C:N ratios in root-affected soil. These shifts were associated with increased 16S rRNA transcripts and denitrifier activities. Straw increased microbial biomass by 124% in the topsoil and at the same time increased root biomass by 125% and number of tillers by 80%. We concluded that microbial activation at the root-straw interface may positively feed back on soil nutrient regulation and plant performance. Further research has to evaluate if plant roots actively prime mining of previously immobilized nutrients in the straw detritusphere or if effects of pathogen suppression and growth promotion are dominating.

Keywords Rhizotron · N immobilization · Nutrient stoichiometry · Nitrification · Denitrification · Wheat straw · Rhizotron sampling · Barley

Abbreviations
NP Treatment with N and P fertilization
WSNP Treatment with wheat straw incorporation and N and P fertilization

† Rüdiger Reichel ru.reichel@fz-juelich.de
1 Institute of Bio- and Geosciences, Forschungszentrum Jülich GmbH, Agrosphere (IBG-3), 52425 Jülich, Germany
2 Helmholtz Zentrum München GmbH, Research Unit for Comparative Microbiome Analysis, Ingolstädter Landstraße 1, 85764 Neuherberg, Germany
3 Chair of Ecosystem Functioning and Services, Institute of Ecology, Leuphana University of Lüneburg, Universitätsallee 1, 21335 Lüneburg, Germany

Introduction
It is increasingly recognized that conventional farming practice and mineral fertilization have negative impact on soil humus stocks (Steinmann et al. 2016) and, less recognized, on microbiological potentials to regulate soil nutrient retention (NO3− leaching, Li et al. 2021) and release to promote plant growth (Malhi et al. 2011). In agricultural soils, due to mineral fertilization, microorganisms are mostly limited by organic C and only secondarily by N or P (Griffiths et al. ...
Plant and soil microorganisms are assumed to compete for N, if C:N ratios of accessible resources are larger than 25 (Amelung et al. 2018). Usually, C:N ratios in agricultural soils are below this threshold, if not temporarily increased, e.g., by incorporation of cereal straw with wider C:N ratios of 50–100. Such straw residues are also characterized by a wide holocellulose: lignin ratio (Wei et al. 2020), which marks the residues as more accessible and faster decomposable to soil microorganisms. Under such conditions, soil microorganisms gain a temporal competitive advantage over plants for available mineral N for biomass production. Therefore, incorporation of straw before winter could be used as a strategy to immobilize excess mineral N in new microbial biomass (Amelung et al. 2018; Reichel et al. 2018). This protects N from leaching during wet winter periods in a manner similar to catch cropping by temporarily immobilizing available soil nutrients in new biomass (Li et al. 2021). Next year’s crops may benefit from the straw-derived microbial N sources, when straw decomposition, and thus C release, subsides and the previously straw-mediated microbial matter is remineralized (Reichel et al. 2018). Organic C oversupply and decay-limiting factors, e.g., high lignin contents as in sawdust, could prolong nutrient immobilization into the next growing season, resulting in critical “N lock” and reduced crop yield (van Duijnen et al. 2018).

Co-application of mineral N and P with straw, however, mitigates “N lock” conditions and additionally favors a more diverse and complex microbial community (Zhao et al. 2019), containing more bacterial and fungal taxa related to C- and N-cycling than in soil without straw amendment (Zhong et al. 2020). Mycorrhizal fungi are plant symbionts and saprotrophic microorganism with the capability to penetrate and take up N from organic patches (Hodge et al. 2001). Yang et al. (2019) reported that bacterial taxa living in the straw detritusphere could favor N availability to plants. They further showed a decrease in plant pathogens with straw amendment as compared to the un-amended control. Suppression of pathogens such as Fusarium wilt in watermelon orchards can be related to positive impacts of wheat straw on the rhizosphere microbial network and diversification of the rhizospheric microbiome (Tang et al. 2020). Straw addition may also prime the disease defenses by an improved uptake of silica from the detritusphere into plant biomass (Rodgers-Gray and Shaw 2000). Overall, N and P fertilization in combination with wheat straw incorporation at a rate of 3.75 t ha$^{-1}$ a$^{-1}$ prior to plowing significantly increased soil organic C, total N, and microbial biomass to which crop yields responded positively (Liu et al. 2010).

However, literature is not conclusive about the effects of a shallow straw detritusphere on microbial regulation of top-dressing fertilizer nutrients and on plant performance. Therefore, and in order to conduct small-scale sampling of soil with minimal disturbance on overall soil C and N dynamics, we developed special rhizotrons with re-sealable ports.

Generally, rhizotron experiments with plant roots growing along a transparent 2D surface are important to improve the mechanistic understanding of dynamic effects, especially if soil and root samplings at a fine temporal and spatial scale can be ensured without massive disturbance or disintegration of the whole soil system. Many methods have been developed to study the root architecture, dynamics, regulatory, and physicochemical mechanisms for mechanistic understanding and modeling to develop sustainable plant management strategies (Hilton et al. 1969; Neumann et al. 2009). More recently, informatics and robotics have led to the invention of automated phenotyping systems such as the GROWSCREEN-Rhizo, which is capable of imaging plant roots and shoots of 60 soil-filled rhizotrons per hour (Nagel et al. 2012). However, most of the rhizotrons were not designed to allow flexible soil sampling or manipulations, e.g., of nutrients or microbial activity, at basically any spot and time during the observation of plant and root development, even if state-of-the-art techniques such as X-ray tomography, magnetic resonance imaging, light transmission imaging, or neutron radiography are applied (Lobet and Draye 2013). Other authors used rather artificial setups with nylon cloth or plates with fine open holes to differentiate technically between different soil and root compartments or to insert micro-suction cups to extract nutrients and other compounds (Kuchenbuch and Jungk 1982; Youssef and Chino 1987; Dessureault-Rompré et al. 2006). In the frame of this study, we used a custom-made rhizotron system with re-sealable sampling ports for a more spatially and temporally flexible soil sampling over time, including different soil depths and soil compartments without substantial disturbance of the plant-soil-microbial system. We used the new rhizotrons to conduct a 90-day greenhouse experiment in which we analyzed soil C:N:P dynamics, carbon dioxide (CO$_2$), and nitrous oxide (N$_2$O) emissions, together with microbial biomass, selected bacterial genes (abundance), and transcripts (activity) in bulk and root-affected soil at multiple times. Our experiment was guided by two hypotheses: (1) shallow wheat straw incorporation in combination with top-dressing mineral fertilization has a more positive effect on plant performance than mineral fertilization alone; (2) positive effects on plant performance can be related to shifts in the extractable C:N:P ratio and increased microbial activity close to the fertilizer-enriched straw detritusphere.

**Material and methods**

**Experimental design**

Eight of the specially designed rhizotrons were used (Fig. 1). Details about the rhizotron design and the sampling
technique are provided in the supplementary material (Fig. S1a, b, c).

A realistic soil profile with a sequence of an A, B, and C horizon was simulated by filling three different soil substrates stepwise into the rhizotrons. Each substrate layer was compacted to the specified bulk density by defined rubber hammer strokes (Table S1). On average, each rhizotron contained 13.3 kg dry substrate with a standard deviation (± SD) between replicates (n = 4) of 1.1%. Soil substrate 1 was obtained as freshly sampled substrate, ordered as “standard soil 2.1” from the Agricultural Investigation and Research Institute LUFA (Speyer, Germany) and used to fill the upper 28 cm of the rhizotron. It was derived from a fallow agricultural Ap horizon without any fertilization or pesticide application during the past 4 years. The soil texture was characterized as loamy sand with 4% clay, 10% silt, and 86% sand. Soil substrate 2 was composed of substrate 1, quartz powder (Ø particle size 90 µm), and quartz sand (Ø particle size 370 µm), mixed at a volumetric ratio of 1:1:6 to form the artificial B horizon at a depth of 28 to 85 cm. Soil substrate 3 contained only quartz sand with an average grain size of 370 µm, forming the C horizon at a depth of 85 to 100 cm. More details about the properties of the substrates can be found in Table S1.

Soil moisture content was slowly adjusted to 30% of the average WHC. The rhizotrons were covered with PARAFILM® M (VWR International GmbH, Darmstadt, Germany) to reduce evaporation and were pre-incubated more than 1 month (8 weeks) to allow for the stabilization of the soil microbial C and N dynamics after rewetting the soil (Fierer and Schimel 2002). Afterwards, the eight rhizotrons were split randomly into a control (mineral fertilizer only, NP) and a wheat straw treatment (mineral fertilizer plus wheat straw, WSNP) group, each comprising four independent replicates.

The practically relevant plant density and fertilizer amount were calculated based on the rhizotron soil surface of 0.00875 m². Each rhizotron received 0.56 g ± 1.1% N fertilizer (YaraBela® SULFAN™: 12% NO₃⁻-N, 12% NH₄⁺-N, 6% S; 12% CaO; YARA GmbH and Co. KG, Dülmen, Germany) and 0.36 g ± 1.1% triple superphosphate (46% P₂O₅ = 20% P; Raiffeisen Waren-Zentrale Rhein-Main eG, Köln, Germany), equivalent to 150 kg N ha⁻¹ and 80 kg P ha⁻¹. Fertilizer grains were evenly distributed at the soil surface, with a distance of 30 mm from each other. The expected N immobilization maximum of 40 kg N ha⁻¹ after wheat straw incorporation in the first month after incorporation was used (Reichel et al. 2018) to adjust the N fertilization to a non-limiting level. In addition, half of the rhizotrons received an equivalent of 4500 kg C ha⁻¹ using 2–5 mm otherwise untreated wheat straw particles (4.0 g per rhizotron, SD ± 0.03%), mixed into the top 0–2 cm of the soil (shallow wheat straw incorporation zone) to manipulate the stoichiometric ratio of C:N:P in this layer.

In contrast to other studies with straw incorporation into the entire plowing layer, we used a shallow incorporation of straw to manipulate the C:N:P stoichiometry close to the mineral fertilizer application zone, which usually is also the soil volume with the largest microbial biomass and activity potential to regulate soil nutrients. Air-dry wheat straw was obtained in 2017 from the Hohenschulen experimental site (Kiel, Germany) and stored under dry conditions at room temperature. The wheat straw had the following characteristics: C = 44.4 ± 0.1%, N = 0.28 ± 0.02%. Two pre-grown summer barley plants (Hordeum vulgare L. cv. LEANDRA ZZ Brau, Hauptsaatbank für die Rheinprovinz GmbH, Germany), corresponding to a density of 228 seed grains per m², were planted at radicle emergence stage. To eliminate
previously reported negative effects of wheat straw on seed germination, e.g., from toxins and pathogenic fungi (Kimber 1973; Nakano et al. 2006; Raaijmakers et al. 2009), barley seedlings were transplanted 20 days after straw application (20 DAF), when most critical straw effects, e.g., of pathogens, most likely had subsided.

The rhizotrons were randomly shifted after each sampling, and the mean of both plants per rhizotron was used for later calculations. The experiment was conducted from January 25 to April 10, 2019, in a greenhouse of the Forschungszentrum Jülich GmbH (Germany; 50°54′31.9″N 6°24′11.0″E). Air temperature in the greenhouse was not controlled and ranged between 1 °C and 18 °C (Fig. S2). A temperature logger with an external sensor was placed in a shaded area near the soil surface to record the air temperature hourly. Relative humidity ranged between 50 and 70% during the experiment (Fig. S2) and was recorded regularly at 12 a.m., using a standard hygrometer. Artificial lighting (SANLight S2W LED lights, SANlight GmbH, Bludenz, Germany) was installed 100 cm above each rhizotron with a spectrum of 400 to 730 nm and a light intensity of 200 μmol m−2 s−1 14.5 h daily. The rhizotrons were watered with 60 mL, unsterile, deionized water (spiked with 0.003 M CaCO3) twice per week. Soil moisture was allowed to fluctuate based on evaporotranspiration changes.

**Soil sampling**

Sampling comprised four independent replicates per treatment. Per replicate, two separate soil samples of 1 cm3 with an average fresh weight of 1.5 g each were sampled. These two separate samples were pooled and then split again. One half was stored at 4 °C for gas emission analysis and extraction of soil nutrients, the other half was used as backup or for molecular biological analyses (−80 °C). Samples from different soil depths were taken at different vertical, but a fixed horizontal location. Samples of bulk soil and root-affected soil were sampled from a fixed vertical, but from different horizontal locations. For the determination of microbial biomass C and N, we destructively sampled the soil at the end of the experiment, as described in more detail below.

To evaluate time effects of a shallow incorporation of wheat straw on distribution of top-dressing fertilizer nutrients, samples were collected from bulk soil 3–5 cm below the treatment application zone 0, 6, 20, 40, 49, and 84 DAF (days after wheat straw amendment and fertilization). Uppermost soil centimeters were omitted to allow an even distribution of dissolving fertilizer along the soil surface and the straw detritusphere.

In order to evaluate the straw effect on the nutrient distribution and N-cycling genes and transcripts in root-affected and bulk soil, sampling was conducted at 3–5-cm soil depth 13 and 29 days after barley transplanting (33 and 49 DAF). Bulk soil was defined as soil from locations without visible roots along the rhizotron window and without hidden roots in the sample. Root-affected soil was defined as soil from locations with visible roots along the rhizotron window. Cut-off roots were removed from root-affected soil before processing. Genes and transcripts of nitrifiers and denitrifiers were assessed at tillering stage 49 DAF (phenological development coding: BBCH 22), which was the earliest time at which visual differentiation between bulk and root-affected soil was possible.

Bulk soil samples were obtained from 3–5 cm, 5–10 cm, 10–20 cm, and 20–30 cm below the soil surface 20 DAF (BBCH 00), 40 DAF (BBCH 22), and 84 DAF (BBCH 33) to evaluate the wheat straw effect on soil C:N:P stoichiometry, and CO2 and N2O gas emission at different soil depths.

Additional bulk soil samples were collected from the soil layers 0–5 cm, 5–10 cm, 10–30 cm (A horizon), and 30–40 cm (B horizon) to quantify the wheat straw effect on microbial biomass C (Cmic) and microbial N (Nmic) at the end of the experiment. These samples were pooled, mixed, and stored at 4 °C until processing.

**Soil physicochemical analyses**

The following sample processing pipeline for greenhouse gas (GHG) and nutrient analyses was applied.

Step 1: Determining the weight of the fresh soil core in a 22-mL gas chromatograph (GC) vial directly after sampling (crimp neck vial N 20, MACHEREY–NAGEL GmbH and Co. KG, Düren, Germany), followed by gas chromatographic analysis of the GHG after 12 h at 20 °C (GC 8610C with electron capture detector, SRI Instruments, CA, USA). The evaporation protection (PARAFILM® M, VWR International GmbH, Darmstadt, Germany) was removed 1 h before crimping the vials gas-tight with butyl rubber septa and aluminum caps (crimp closure N 20, septa hardness 50° shore A, MACHEREY–NAGEL GmbH and Co. KG, Düren, Germany). Twenty-milliliter ambient air was injected with a syringe into each sealed GC vial at the beginning to have enough gas volume for repeated sampling by the GC autosampler after 1, 4, and 7 h of closed incubation. Ambient air was sampled before the vial preparation as time point zero. Each measurement reduced the gas volume in the vial by 3.5 mL, which was considered by stepwise reduction of the gas volume in the calculation of CO2-C and N2O-N g−1 h−1 (Reichel et al. 2018).

Step 2: After GHG measurement, vials were opened and covered with a perforated aluminum foil, then frozen at −20 °C, and subsequently freeze-dried. Then, the weight of the dry soil core in the 22-mL GC vial was determined to get dry-to-fresh soil mass ratios for later calculations. Gravitometric water content was determined by dividing the water
content by the soil dry mass, multiplied by 100. Freeze-dried samples were stored closed gas-tight at 4 °C until performing the soil extractions as described below.

Step 3: The standard method for soil nutrient extraction (VDLUFA 1991) was modified as follows: nutrient extraction of freeze-dried soil cores was performed in 50-mL falcon tubes (VWR International, Darmstadt, Germany), using 0.01 M CaCl₂ solution and a dry soil-to-solution ratio of 1:20 (w/v). Soil samples with an average dry weight of 1.5 g were dispersed in 30 mL of the 0.01 M CaCl₂ solution and mixed for 4 h by over-head shaking (Heidolph Reax 2, Heidolph Instruments GmbH and CO. KG, Schwabach, Germany) at a speed of 20 turns min⁻¹. Soil and solution were separated by centrifugation at 690 × g for 30 min. Twenty milliliters of the supernatant were dispersed in 30 mL of the 0.01 M CaCl₂ solution and a dry soil-to-solution ratio of 1:20 (w/v). Soil samples with an average dry weight of 1.5 g were dispersed in 30 mL of the 0.01 M CaCl₂ solution and mixed for 4 h by over-head shaking (Heidolph Reax 2, Heidolph Instruments GmbH and CO. KG, Schwabach, Germany) at a speed of 20 turns min⁻¹. Soil and solution were separated by centrifugation at 690 × g for 30 min. Twenty milliliters of the supernatant was filtered through 0.45-µm polypropylene membrane filters (disc size 25 mm; Macherey–Nagel, Düren, Germany). The solution was stored at 4 °C until the determination of the pH, dissolved organic C (DOC), mineral N (Nₘ⁻ = sum of exchangeable NH₄⁺-N and NO₃⁻-N), and 0.01 M CaCl₂-extractable phosphorus (P₉CaCl₂). To check the influence of freeze-drying on the compound extractability, a laboratory pre-test was performed on fresh and freeze-dried soil cores. Results showed that freeze-drying significantly (t test, p < 0.05) increased the DOC content by 109%. The concentration of H⁺ (pH), Nₘ⁻, and P₉CaCl₂ was not significantly affected by freeze-drying of the specific soil. Based on this, only the DOC results after extraction of freeze-dried soil were corrected by a factor of 0.48.

pH values were determined using a pH electrode and a multimeter (multi 340i pH meter, Xylem Analytics Sales GmbH and Co., Weilheim, Germany). Eight milliliters of the fresh soil extract was transferred into a 24-mL screw neck TOC vial (Macherey–Nagel, Düren, Germany) and made up with distilled water to a total volume of 20 mL (dilution factor of 2.5) before analyzing DOC with a TOC analyzer (TOC-VcPH, Shimadzu, Japan). Twelve milliliters of the final soil extract was transferred into a 20-mL polyethylene vial with a quick closure cap (PerkinElmer Inc., MA, USA). Nitrate and exchangeable NH₄⁺ were measured by ion chromatography (Dionex DX-500, ThermoScientific, MA, USA). Phosphorus (P₉CaCl₂) was determined by inductively coupled plasma optical emission spectrometry (iCAP™ 7600 ICP-OES Analyzer, ThermoScientific, MA, USA).

Leachate was collected if available, frozen at −20 °C, and combined to a bulk sample to analyze for the nutrients as described above (i.e., step 3).

**Microbial analyses**

Soil sampled at the end of the experiment from different soil depths was used to determine the microbial biomass C (Cmic) and N (Nmic) by the chloroform-fumigation extraction based on Joergensen (1996). Forty milliliters of the soil extract was centrifuged at 690 × g for 15 min before filtering through a 0.45-µm polypropylene membrane filter (47-mm diameter, Macherey–Nagel, Germany). Ethanol-free chloroform for liquid chromatography was used (LiChrosolv®, Merck Chemicals GmbH, Darmstadt, Germany). The closed desiccator was incubated at room temperature for 24 h. DOC and dissolved N were measured using a TOC analyzer (TOC-VePH + TNM-1 + ASI-V, Shimadzu, Japan). Cmic and Nmic were calculated as difference of DOC and dissolved N by subtracting non-fumigated blank from fumigated sample and correction by the factors kEC 0.45 and kEN 0.40, as reported by Joergensen (1996).

**Abundance and activity of nitrifiers and denitrifiers**

DNA and RNA were extracted from 0.5 g of frozen soil (−80 °C), using a co-extraction protocol modified according to Töwe et al. (2011). The co-extracts were quantified using a Nanodrop 2000 spectrophotometer (ThermoFisher Scientific, USA) and stored at −80 °C until further analysis.

| Target     | Cycle no | Thermal profile | Primer | Reference                  |
|------------|----------|-----------------|--------|----------------------------|
| 16S (V4/V5 region) | 35       | 95 °C–45 s/58 °C–45 s/72 °C–45 s | 16F, 16R | (Bach et al. 2002)        |
| nirK       | 5*       | 95 °C–15 s/63 °C–30 s/72 °C–30 s | nirK876, nirK5R | (Henry et al. 2004; Braker et al. 1998) |
| nirS       | 40       | 95 °C–15 s/58 °C–30 s/72 °C–30 s | nirScd3a, nirS3cd | (Michotey et al. 2000; Throßbäck et al. 2004) |
| nosZ       | 5*       | 95 °C–15 s/55 °C–30 s/72 °C–30 s | nosZ2F, nosZ2R | (Henry et al. 2006)         |
| amoA AOA   | 40       | 94 °C–45 s/55 °C–45 s/72 °C–45 s | amo19F, CrenamoA616r48x | (Leininger et al. 2006) |
| amoA AOB   | 39       | 94 °C–60 s/58 °C–60 s/72 °C–60 s | amoA1F, amoA2R | (Rotthauwe et al. 1997)     |

*Represents touchdown PCR
The co-extracts were divided into two tubes and labeled as DNA and RNA for further analysis. DNA digestion was performed on the RNA samples using the RNA Clean and Concentrator-5 kit (ZymoResearch, USA), following the protocol instructions. To assess the success of the DNase treatment, a PCR was performed targeting the V4/V5 region of the 16S rRNA gene. RNA purification was then carried out using the same kit described above, and the quality and quantity of the purified RNA were determined using the DNF-471 Standard Sensitivity RNA kit on a Fragment Analyzer Instrument (Agilent Technologies, California, USA). cDNA synthesis was performed using the SuperScript IV VILO master mix kit (ThermoFischer Scientific, USA) following the manufacturer’s instructions.

Absolute quantification of the 16S rRNA gene, as well as marker genes for denitrification (nirK, nirS, and nosZ) and nitrification (amoA for ammonia-oxidizing archaea (AOA) and bacteria (AOB)) for both DNA and RNA was done using real-time quantitative PCR (q-PCR) on a 7300 Real-Time PCR System (Applied Biosystems, MA, USA). The primers used are described in Table 1. The PCR reaction mix contained: 1× Power SYBR™ Green PCR Master Mix (Applied Biosystems, MA, USA), 7.5 pmol of each primer (for the amoA gene of AOB) and 5 pmol of each primer for the other genes, 0.06% bovine serum albumin (BSA), 2.5% DMSO (for nirK and nirS genes), 1 µL of template DNA/cDNA and DEPC water to a final volume of 25 µl. The optimal dilution for the DNA and cDNA was determined by testing for possible inhibitory effects on qPCR amplification caused by co-extracted humic substances. A dilution of (1:100 v/v) of the DNA and cDNA was selected for amplification (data not shown). All PCRs involved an initial activation step at 95 °C for 10 min and 1 cycle of product melting at 95 °C for 15 s, 60 °C for 30 s, and 95 °C for 15 s. Melting curves were used to check the dissociation pattern of the amplified products. Serial dilutions (ranging from 10^4 to 10^7 gene copies-µl^-1) of plasmids containing the tested genes in known concentrations were used to generate the standard curve. The amplification efficiencies were calculated using the formula Eff = [10^(−1/slope) − 1] and gene copy numbers normalized to grams of soil.

**Plant sampling and analyses**

Relative leaf greenness (a proxy for chlorophyll content) was assessed in order to evaluate the wheat straw treatment effect on barley plants as single-photon avalanche diode (SPAD) value 51, 56, 68, 77, and 90 DAF, using a chlorophyll meter (SPAD-502Plus, Konica Minolta, Inc., Tokyo, Japan; based on Süß et al. 2015). To reduce the variability of the SPAD values within each plant and along each leaf, the measurement was performed on the second fully unfolded leaf from the tip of the main stem to estimate the actual chlorophyll status. The instrument was placed at four spots decentral to the leaf primary vein to assess the SPAD median value.

The height of each shoot was measured from the base to the stem below the uppermost main shoot leaf to access the shoot height, leaf number, and tiller number 56, 68, 77, and 90 DAF. Leaf and tiller number of each plant were assessed by manual counting, including all living and dead leaflets.

Root and shoot were separated by cutting the shoot at the base to determine the shoot and root biomass (for roots after thorough washing with tap water) 90 DAF. Plant materials were dried at 60 °C until reaching a constant mass for determining the root and shoot in g plant^-1 dm. After ball-milling (MM 400, Retsch, Germany), the total C and N content of the finely ground plant material was measured with an elemental analyzer (Vario EL, Elementar, Germany). Total P content was measured after microwave digestion with 2 mL HNO3 (65%) and 1 mL H2O2 (30%) at 800 W, 20 min gradient from room temperature to 160 °C, and a final holding time of 15 min (MARS 5, CEM GmbH, Kamp-Lintfort, Germany; based on Nischwitz et al. 2017). Digested samples were made up to 14 mL with ultra-pure water. The obtained solution was diluted 1:20 with ultrapure water before measurement with ICP-OES (iCAP™ 7600 ICP-OES Analyzer, ThermoScientific, MA, USA). The results were used to calculate the root-to-shoot and C:N:P ratios (Table S6).

**Statistical analyses**

Results are presented as mean value ± standard deviation (± SD) of four independent replicates per treatment (control and wheat straw treatment). Statistics were performed using SPSS Statistics 21 (IBM, Ehningen, Germany) and the R software package (V.3.6.0; R Core Team 2020). The data were analyzed using the Shapiro–Wilk test to check if the normality assumption was valid.

One-way analysis of variance (ANOVA) was used to test if straw addition affected plant and microbial parameters. Two-way ANOVA models were used to evaluate the effect of straw addition, time, and their interactions on soil parameters like DOC, Nmin, NO3−, exchangeable NH4+, PCaCl2, CO2, and N2O, as well as on plant parameters such as SPAD, shoot height, and leaf number. Three-way ANOVA models were used to test if straw addition, time, soil depth or root-affected vs. bulk soil, and their interactions affected the soil parameters. Depending on the statistical assumption validity, we performed post hoc comparisons using Tukey-B or t test. Otherwise, the Wilcoxon test, Kruskal–Wallis test, or Mann–Whitney U test was used. More information is presented in corresponding tables or figures.
Results

Prior to the wheat straw and fertilizer application, C:N:P stoichiometry (molar ratio of the parameters of extractable DOC:N\text{min}^{1+}\text{P}_{\text{CaCl}_2}) of the rhizotron soils was statistically not different. Gravimetric moisture content decreased in both treatments over time in the soil layer 3–5 cm ($F = 45.9$, $p < 0.001$; Table S2) and showed a declining gradient from top to deeper soil layers ($F = 19.2$, $p < 0.001$; Table S3), but no difference between bulk and root-affected soil (Table S4). Bulk leachate samples of the WSNP treatment (88 mL) contained about 14% more DOC (1.2 mg), 3% less $N_{\text{min}}$ in the form of $\text{NO}_3^{−}$ (2.8 mg), but 9% more $P$ (2.4 mg) compared to the NP treatment (92 mL).

Dynamics of extractable C:N:P in soil after wheat straw application

In the top soil layer (3–5 cm), time and wheat straw amendment showed interactive effects on the DOC concentration ($F = 8.3$, $p < 0.001$; Fig. 2A). In addition, for DOC both factors also interacted with the soil compartment ($F = 5.5$, $p < 0.05$; Table S4). Wheat straw incorporation increased the DOC concentration by 79% ($p < 0.05$) in the WSNP treatment at 6 DAF, followed by a rapid decline to the level of the NP treatment at 20 DAF (Fig. 2A). In the WSNP treatment, DOC concentrations were increased in the root-affected compared to the bulk soil at 49 DAF (Table S4).

Time ($F = 188.4$, $p < 0.001$; Fig. 2), wheat straw treatment ($F = 16.8$, $p < 0.001$; Fig. 2), and soil depth ($F = 22.2$; $p < 0.001$; Table S3) had an impact on the soil $N_{\text{min}}$ concentration, while the soil compartment only interacted with the factors time and treatment ($F = 10.8$, $p < 0.01$; Table S4).

Soil with wheat straw incorporation contained about 25% less $N_{\text{min}}$ (in the soil layers 3–5 cm: $\text{NO}_3^{−}$ + exchangeable $\text{NH}_4^{+}$; 5–10 cm: $\text{NO}_3^{−}$) compared to the NP treatment (Fig. 2B, Tables S2 and S3). In the root-affected soil of the WSNP treatment, 33% less exchangeable $\text{NH}_4^{+}$ was observed compared to bulk soil at 49 DAF (Table S4).

Time and the wheat straw amendment had only small effects on extractable $P_{\text{CaCl}_2}$ (Table S2) in combination with the factors soil depth ($F = 4.2$, $p < 0.01$; Table S3) and soil compartment ($F = 4.9$, $p < 0.05$; Table S4). Concentration of $P_{\text{CaCl}_2}$ was increased in the 3–5 cm and 5–10 cm soil layer compared to the lower soil layers (Table S3). Wheat straw amendment increased the concentration of $P_{\text{CaCl}_2}$ in the WSNP compared to the NP root-affected soil at 33 DAF (Table S4).

Microbial response to dynamics of extractable C:N:P in soil

Time and straw treatment affected $\text{CO}_2$ emission ($F = 12.5$, $p < 0.001$, Fig. 2C), interacting with soil depth ($F = 2.5$, $p < 0.05$; Table S4), but not with the factor soil compartment. Straw incorporation initially increased $\text{CO}_2$ emissions (Fig. 2C). More $\text{CO}_2$ was emitted from the 3–5 cm soil layer compared to the 20–30 cm soil layer at 20 and 40 DAF (Table S3).

Emission of $\text{N}_2\text{O}$ changed with time ($F = 201.1$, $p < 0.001$) and treatment ($F = 6.1$, $p < 0.05$, Table S2), with a slight interaction of treatment and soil depth ($F = 3.2$; $p < 0.05$). $\text{N}_2\text{O}$ emission was significantly lower in the WSNP compared to the NP treatment at 84 DAF. Occasionally, more $\text{N}_2\text{O}$ was emitted from the NP compared to the WSNP treatment also from greater soil depths (Table S3).

![Fig. 2](image-url) Wheat straw effects over time ($p < 0.001$) on physicochemical soil parameter mean values of the NP (control) and the WSNP (wheat straw) treatment in the soil layer 3–5 cm (below the treatment application zone) zero days after wheat straw amendment and fertilization (0 DAF), 6, 20 (transplanting of barley, black arrow), 40, 49, 84 DAF. (A) Dissolved organic C (DOC), (B) extractable $N_{\text{min}}$ (exchangeable $\text{NH}_4^{+}$-N and $\text{NO}_3^{−}$-N), and (C) carbon dioxide emission ($\text{CO}_2$ emission). Units are displayed at the y-axis. Regressions with $R^2$ are polynomial. Asterisks indicate significant differences between the treatments at $p \leq 0.05$. More details in Table S2
There was no obvious wheat straw treatment effect on the abundance of 16S rRNA genes in bulk and root-affected soil at 49 DAF (Fig. S3A), indicating comparable abundance of bacteria. However, in the root-affected soil of the WSNP treatment the abundance of 16S rRNA was significantly increased compared to the NP treatment at 49 DAF (Fig. 3A), which points to an increased activity of bacteria in the root-affected soil of the WSNP treatment. An initially increased abundance of nirK genes and nosZ transcripts in bulk soil (0 DAF) disappeared or reversed at 49 DAF, assuming no systematic bias on the experiment (Fig. 3F, S3E).

Fig. 3 Wheat straw effects on (A) overall bacterial activity (16S rRNA transcripts), (B) ammonia-oxidizing activity of archaea (AOA), (C) ammonia-oxidizing activity of bacteria (AOB), (D) nitrite reductase activity (nirS), (E) nitrite reductase activity (nirK), and (F) nitrous oxide reductase activity (nosZ) in bulk and root-affected soil of the NP (control) and the WSNP (wheat straw) treatment zero days after amendment and fertilization (0 DAF) and 49 DAF (29 days after barley transplanting) in soil dry matter (dm). Significances between the treatments are illustrated with: ns \((p > 0.05)\), * \((p < 0.05)\), ** \((p < 0.01)\), and *** \((p < 0.001)\).
Compared to AOA, the abundance of amoA transcripts of AOB was lower in the WSNP bulk soil at 49 DAF ($p = 0.057$). This might point to a negative effect of WSNP on bacterial ammonia oxidizers, while no such shift occurred in soil of the NP treatment or in the WSNP root-affected soil. This was accompanied by soil stoichiometric shifts towards larger C:P and C:N ratios in the root-affected soil, but lower N:P ratios in the WSNP treatment at 49 DAF (Fig. 4A–C).

In root-affected soil of the WSNP treatment, the abundance of nirS and nirK transcripts, as well as of nosZ genes and transcripts, was larger compared to the NP treatment at 49 DAF (Fig. S3 and Fig. 3D–F), indicating a strong increase of denitrifiers capable of complete denitrification both in abundance and activity as a result of the WSNP addition. These shifts in the root-affected soil after wheat straw incorporation occurred simultaneously with shifts of the soil C:N:P stoichiometry at 49 DAF (Fig. 4A–C).

C_{mic} (F = 41.4, $p < 0.001$; Fig. 5A) and N_{mic} (F = 63.3; $p < 0.001$; Table S5) were affected by treatment and soil depth. Wheat straw mostly increased the C_{mic} by 124% in the first 5 cm of the WSNP compared to the NP soil at 90 DAF. Even at a soil depth of 10 to 30 cm, the WSNP treatment revealed an increased C_{mic} compared to the NP control (Table S5).

**Plant responses to dynamics of extractable C:N:P in soil**

The relative leaf greenness (SPAD value) was affected by the factor time (F = 6.6, $p < 0.01$), indicated by an increase in SPAD values of 39% and 20% in the NP and WSNP treatment between 51 and 90 DAF, respectively (Table S6). Besides the leaf number, tiller number was increased (by 1.8 tillers plant$^{-1}$) in the WSNP compared to the NP treatment at 90 DAF (Fig. 5B). Root biomass per plant was increased by 125% in the WSNP compared to the NP treatment (Fig. 5B, Table S6). Roots of the WSNP treatment contained significantly less C (17%) compared to the NP treatment, lowering the root C:N ratio. Shoot C and N was not significantly different in the WSNP compared to the NP treatment (Table S6).
Discussion

Overall, we aimed to evaluate if a shallow wheat straw incorporation in combination with top-dressing mineral fertilization has a more positive effect on plant performance due to beneficial changes in microbial activity and nutrient stoichiometry than mineral fertilization alone. In order to allow a small-scale soil sampling with minimal effects on overall soil C and N dynamics, we developed special rhizotrons with resealable ports.

Improved plant performance after straw incorporation with mineral fertilization

Wheat straw addition significantly increased microbial biomass (Cmic and Nmic, Table S5, Fig. 5A), indicating microbial N immobilization, without a restricted nutrient availability to plants as frequently reported (e.g., Cheshire et al. 1999). In soils with low nutrient accessibility, organic C amendments can lead to a competitive microbial nutrient immobilization and “N-lock” effects on plant growth and yield (Ganry et al. 1978; Hauggaard-Nielsen et al. 1998; Elazab et al. 2016). However, incorporation of accessible organic C can also lead to microbial mining of N from soil organic matter (Fontaine et al. 2011), which could be beneficial for plant nutrition after decay of these microorganisms at later times (Reichel et al. 2018). In the present study, straw significantly reduced the availability of mineral N (NH4\(^+\) and NO3\(^–\)) in soil (Tables S2–4), however, only to a level without signs of N deficiency of plants (chlorophyll content, Table S6). In a mesocosm experiment, van Duijnen et al. 2018 found no effect of wheat straw, but negative effects of sawdust addition on barley biomass and grain yield. Our results clearly showed that a shallow incorporation of wheat straw in spring with adequate mineral fertilization does not limit nutrient supply and growth of plants.

This study showed that a straw incorporation in spring keeps a substantial amount of nutrients bound in the microbial biomass in the upper soil layers, making them potentially more accessible to young plant roots if released in the right amount and at the right time. The nutrient retention effect was indicated by consistently increased microbial biomass (Fig. 5A), approximately 25% lower mineral N concentration, and 3% reduced NO3\(^–\) leaching in the straw treatment (Fig. 2B). Our study also indicates that the wheat straw treatment-mediated N:P shifts in the bulk soil may temporarily lower the abundance of ammonia-oxidizing bacteria and related NO3\(^–\) loss from soil (AOB, Fig. S3C). Lower abundances of AOB in bulk soil appear to be related to their low competitiveness for exchangeable NH4\(^+\) compared to other N-immobilizing soil microorganisms (Norman and Barrett 2014). Our data also indicate that the presence of roots might override this straw-mediated effect of N:P shifts on ammonia-oxidation in bulk soil (Fig. S3C). Nonetheless, evidence from the literature suggests that organic soil amendments such as wheat straw protect nutrients from leaching during wintertime through incorporation in microbial biomass (Van Duijnen et al. 2018; Liu et al. 2021), potentially making them more accessible for root uptake in the following year. Young plants with shallow root systems could especially benefit from this type of microbially mediated nutrient storage near the soil surface. In addition, the present study shows that young crop plants benefit from a shallow incorporation of wheat straw with mineral fertilization in spring, proving our first hypothesis.

Straw-mediated nutrient shifts and microbial activation in root-associated soil

Besides the possibility of a colonization of roots with beneficial microorganisms that outcompete pathogens (Iswandi et al. 1987; Schulz-Bohm et al. 2018; Tang et al. 2020), net positive effects on plant performance might also relate to...
an improved re-mineralization of previously immobilized nutrients.

Close interactions between wheat straw and barley roots (Fig. 4S) caused shifts towards a greater C:P and C:N ratios in the root-affected soil (Fig. 4A, C), which significantly enhanced microbial activity (16S rRNA transcripts, Fig. 3A). According to the plant-microbial interactions framework of Čapek et al. (2018), wheat straw may have optimized the difference between the nutrient limitation levels of plants (N:P) and soil microorganisms (C:N:P), resulting in more cooperative (beneficial) than competitive plant-soil microbial interactions. Lemanski et al. (2019) found additional evidence that reduced microbial C limitation during the growing season could improve crop yields. Literature shows that temporal and factorial N:P availability are expected to affect barley growth performance by altering root system responses and C investment in nutrient foraging (Kumar et al. 2020; van Duijnen et al. 2021).

Furthermore, surplus of C from living roots and residues leads to microbial activation, and as a result, microorganisms might start to decompose straw-derived soil organic matter to acquire nutrients (Kumar et al. 2016; Clocchiatti et al. 2021). Fontaine et al. (2004) also showed that increased supply of readily available C could accelerate mineralization of soil organic matter to fulfill microbial nutrient demand. Easy available C in the form of glucose, mimicking root C exudates, might regulate the nutrient release by microbial mining in soil organic matter (Shahbaz et al. 2018).

In addition, the significant increase in total microbial biomass C (C_{mic}, Fig. 5A) in contrast to bacterial 16S rRNA gene (Fig. S3A) might indicate the involvement of root-associated fungi. It is known that saprotrophic fungal biomass increases after the addition of cellulose-rich materials to the soil. Clocchiatti et al. (2021) showed increased biomass and activity of mycorrhizal fungi after the addition of C-dominated amendments such as sawdust. Wheat straw promoted fungal growth under N-unlimited soil conditions (Henriksen and Breland 1999), as in the present experiment, by creating nutrient patches that were attractive to root and fungal foraging (Cheng et al. 2016). Mycorrhizal fungi as potential plant symbionts are capable of enhancing decomposition of organic patches, potentially supporting the plant nutrient supply (Hodge et al. 2001). These fungi possess strong exoenzymes of which phosphatase activity in soil of the straw treatment could explain the increased P availability (Table S4; Joner and Jakobsen 1995; Akhtar et al. 2018). In the initial decomposition phase of wheat straw, a considerable proportion of the microbial biomass is likely related to microorganisms that are rapidly outcompeted and die after depletion of organic C (Reichel et al. 2018). This newly formed microbial necromass is suggested as nutrient reservoir for microorganisms and plants after remobilization by exoenzymes (Shabaz et al. 2017). We assume that C-rich amendments as applied in this study help to build up such organic nutrient reserves from which microorganisms and plants can later benefit (Fig. S4, Clocchiatti et al. 2021).

We could show that N cycling was enhanced by root activity in root-affected soil near the straw detritusphere, which appeared to be associated with greater denitrifying activity (Fig. 3D–F). Yang et al. (2019) suggest that wheat straw has the potential to stimulate soil N cycling in a manner beneficial to plant growth. In addition, our study suggests that root interaction with straw favors complete denitrification to N_{2} rather than N_{2}O (Fig. 3D–F; Fig. 4D), which could be considered indicative of a more closed N cycle than in bulk soil. New primers for Clade I nosZ might even detect a higher number of responding taxa than those used in the present study (Table 1; Zhang et al. 2021a, b). Zhou et al. (2020) relate a more complete denitrification to straw-induced shifts in the C:N ratio, and it is well known that shifts in C:N:P ratio have a major impact on denitrification and N_{2}O:N_{2} emission ratio (Smith and Tiedje 1979; Firestone et al. 1980; Paul and Beauchamp 1989; Mehnaz and Dijkstra 2016).

In accordance with our second hypothesis, the results of this study suggest that positive effects on plant performance are related to straw-mediated shifts of extractable C:N:P ratio, microbial activity, and N cycling in root-affected soil.

**Conclusion**

Under conditions as applied in this study, we could show that a shallow wheat straw application may improve the microbial regulation of top-dressing fertilizer nutrients with benefits on plant growth in spring. We could validate the hypothesis that shallow wheat straw incorporation with mineral nutrients has a more positive effect on plant performance than mineral fertilization alone. The in-house developed rhizotrons with resealable soil sampling ports made it possible to take samples at small scale along a 2D soil profile and during plant growth with minimal disturbance, which provided insight in the overall C and N cycle processes and plant-soil-microbial interactions. Therefore, we have been able to link plant performance to changes in extractable C:N:P, stimulated microbial activity, and increased N cycling in root-affected soil, thereby validating hypothesis two. Based on our results, we think that plants roots are able to optimize their nutrient supply by targeted root exudation of C as a prerequisite to stimulate re-mineralization of nutrients previously immobilized in straw-mediated microbial biomass. Further research has to evaluate if plant roots actively prime mining of previously immobilized nutrients in the straw detritusphere or if effects of pathogen suppression and growth promotion are dominating.
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Author contribution Rüdiger Reichel and Nicolas Brüggemann designed the rhizoboxes and the experiment. Rüdiger Reichel prepared the material, collected, and analyzed the data. Zhijie Li supported the experiment and sampling. Nitrification and denitrification genes were analyzed and interpreted by Catherine Wambui Kamau, Viviane Radl, and Michael Schloter. Amit Kumar and Vicky Temperton supported the planning of the experiment and plant nutrient analyses. The first draft of the manuscript was written by Rüdiger Reichel, and all authors revised and commented on previous versions of the manuscript. All approved the final manuscript.

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