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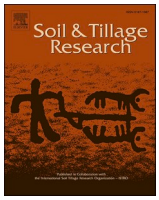

Performance of different wheat varieties and their associated microbiome under contrasting tillage and fertilization intensities: Insights from a Swiss long-term field experiment

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ABSTRACT

Winter wheat is an important global cereal crop. However, conventional farming practices, characterised by intensive tillage and high fertilizer inputs, pose significant threats to the environment. In response, more conservative management practices are being applied aiming to maintain wheat production while promoting a beneficial microbiome. Here, we evaluated the suitability of three different wheat varieties for less intensive agricultural systems, focusing on reduced tillage and fertilizer intensity. The study was conducted over two consecutive years in a Swiss long-term field experiment comparing conventional versus reduced tillage and full fertilization versus half fertilization. In addition, we investigated the composition of plant-associated microbial communities using amplicon sequencing of phylogenetic marker genes, specifically targeting bacteria and fungi in rhizosphere samples and fungi in root samples. Our results revealed that in our study wheat variety most strongly predicted grain yield and quality, independent of tillage and fertilization intensity. Specifically, wheat varieties demonstrated higher yields and N uptake in plots subjected to conventional ploughing and full fertilization compared to those under reduced tillage and half fertilization. We found no significant effect of wheat variety on the composition of microbial communities. However, tillage emerged as the primary factor influencing microbial community composition in the rhizosphere, while fertilization intensity significantly impacted fungal communities in the root system. These findings underscore the complex interplay between agronomic practices, plant genetics, and microbial dynamics in agroecosystems, emphasizing the need for holistic and adaptive approaches and their further development to ensure sustainable crop production.

1. Introduction

Intensive plough-based soil management and fertilization are known to negatively impact soil systems by disrupting soil structure, reducing soil organic matter (SOM) content and shifting soil moisture retention and soil biodiversity, ultimately affecting soil functioning (Degrune et al., 2016; Haddaway et al., 2017; Kraut-Cohen et al., 2020; Li et al., 2019; Peng et al., 2020). To reduce the negative impacts of conventional agriculture, less intensive management practices can be applied including minimum soil tillage and reduced application of fertilizers (Kassam et al., 2019). As studies have shown, reducing tillage intensity has the potential to increase topsoil SOM, reduce soil erosion and increase soil biodiversity (Haddaway et al., 2017; Holland, 2004; Lal, 2009). However, effects on crop productivity have shown variable effects depending on the farming system and the local pedoclimatic conditions. Organic farming in particular favours reduced tillage over no-tillage because of the ability to mechanically control weeds, as synthetic pesticides are banned. A meta-analysis found a 7 % yield reduction in organic reduced tillage systems compared to ploughed systems (Cooper et al., 2016). However, this yield difference was not consistent across studies, and similar or higher yields have also been reported (Krauss et al., 2020; Peigné et al., 2014). Thus, further research is needed to better understand and predict the effectiveness of reduced tillage in organic farming. An important aspect of this research is the

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search for crop varieties adapted to these systems. Crop growth in reduced tillage systems can be impaired by the increased soil density in the former plough layer (Krauss et al., 2022). Therefore, the use of crop varieties with different root systems and rooting characteristics could mitigate potential yield limitations in these systems. Despite this potential, there are currently no studies comparing different crop varieties side by side in reduced tillage organic systems.

Soil microorganisms are responsible for multiple agroecosystem services including nutrient cycling, pest and disease control and thus soil health and fertility (Bender et al., 2023; Delgado-Baquerizo et al., 2016; Wagg et al., 2021). Several studies have documented the beneficial impacts of reduced tillage practices on increasing the abundance of soil microorganism (Chen et al., 2020; Essel et al., 2019; Helgason et al., 2009; Mathew et al., 2012; Morugán-Coronado et al., 2022). Through the disruption of fungal mycelia, intensive tillage is suspected to negatively impact fungal communities especially the symbiotic arbuscular mycorrhizal fungi (AMF). Several studies reported decreases in AMF abundance and diversity under conventional tillage (Brito et al., 2012; Jansa et al., 2002; Oehl et al., 2005). In contrast, a recent meta-analysis found that the response of soil microbial diversity to tillage is highly variable and depending on climatic conditions and soil properties (de Graaff et al., 2019): While minimum tillage increased bacterial diversity by 7 %, there was no significant effect on fungal and AMF diversity compared to ploughed systems due to the high variability across studies. So far, all molecular studies investigating the impact of tillage intensity on AMF community structure used general fungal primers instead of AMF-specific ones. Consequently, only a very small proportion of AMF inhabiting the soil was captured, which makes it difficult to study their diversity and to draw conclusions about the impact of tillage on their community structure. Furthermore, remarkably little attention has been paid to the study of fungi, particularly AMF, within root systems under different tillage systems. The predominant focus of existing research has been on rhizosphere communities, as summarized above, leaving a notable gap in our understanding of fungal dynamics within roots under different tillage systems.

Especially in organic farming systems, microbial communities are of key importance for crop nutrient provisioning as they are required to convert organically bound nutrients within organic fertilizers into plant-available forms. However, studies investigating microbial communities in organic reduced tillage systems are widely missing.

In the present study, we investigated how reduced tillage and fertilizer input in organically managed systems affect the performance of winter wheat varieties and the diversity of soil microbial communities. In two consecutive years, three winter wheat varieties (Wiwa, Apache and Allez-y) were grown in a long-term tillage trial comparing conventional tillage and reduced tillage under full slurry and half slurry fertilization. We assessed a range of agronomic traits of winter wheat and the diversity and structure of rhizosphere-associated microbial communities by amplicon sequencing of phylogenetic marker genes. For fungi, we used primers designed to target AMF and also examined root samples to understand how the factors studied might influence the root colonisation potential of fungal communities. We hypothesized that yield and quality traits differ between wheat varieties cultivated under different management practices due to variety-specific differences in root structure influencing nutrient uptake efficiencies. Further, we hypothesized different management practices and wheat varieties to affect microbial communities leading to a distinct diversity and structure of root and rhizosphere communities in the different tillage and fertilization systems.

2. Material and methods

2.1. Site description and set-up of the field experiment

The study was performed in 2019 (October 2018 to July 2019) and 2020 (October 2019 to July 2020) using the Swiss long-term tillage experiment on the organic farm “Schlatthof” (Aesch, Basel-Land, Switzerland, 47°28’51.6"N 7°34’33.6" E). The site is located at 349 m above sea level and the soil type is characterised as Luvisol with a silty loam texture (20 % clay, 52 % silt, 28 % sand). In 2019, the average temperature throughout the vegetation period was 10.5°C, and 11.1°C in 2020. The total precipitation reached 602 mm and 623 mm in 2019 and 2020, respectively. The experiment, set up in 2010, implements a four-year crop rotation (grass-clover, silage maize, field bean and winter wheat) and is organized in a split-plot design with fertilization intensity nested in tillage as factors and four field replicates. The tillage treatments are conventional tillage (“CT”: annual plough, maximum ploughing depth: 20 cm) versus reduced tillage (“RT”: stubble cleaner or wide sweep chisel or Weco-Dyn, maximum depth: 10 cm). The fertilization treatments included in the current study are two out of five fertilization treatments namely slurry 1 (“N60”: approx. 60 kg total N (TN) ha^{-1} $year^{-1}$) and slurry 2 (“N120”: approx. 120 kg TN ha^{-1} $year^{-1}$). A detailed description of the long-term field trial is given in Grosse et al. (in prep.). The fertilization sub-plots were split to evaluate the performance of three different winter wheat varieties over a two-year period (Figure S1), each with unique traits as specified by the breeders: (i) Allez-y, a French conventional cultivar, rather resistant to water shortage, but sensitive to root diseases (Semences de France, France), (ii) “Apache”, a French conventional cultivar resistant to water shortage and root diseases (Semences de France, France), and (iii) Wiwa, an variety for organic farming systems (UFA Samen, Switzerland). The difference in root disease resistance between the two conventional varieties suggests differences in their rooting systems, making them interesting candidates to compare side by side under different tillage and fertiliser regimes, alongside the organic variety Wiwa. In both years, sowing was done with 425 grains per m^2 at the end of October 2018 and 2019, respectively. Seeds were sown at a depth of approx. 5 cm.

2.2. Sample collection

In spring, before the first fertilization (March 27, 2019, and March 04, 2020, respectively), soil samples were taken with a soil auger (3 cm Ø) from the top 20 cm of soil to obtain approximately one kg of soil. We determined soil pH, total and organic soil carbon (C) and nitrogen (N), phosphorous (P), potassium (K), magnesium (Mg) and calcium (Ca) once in 2019 and soil mineral N (N_{min}) in both years (Table S1). At anthesis, we also measured soil water content (Figure S2) and assessed weed infestation, categorizing it by percentage (Figure S3).

At anthesis (June 25/26 2019 and June 2/3, 2020), rhizosphere soil and roots were collected: three or four wheat plants were excavated from a depth of 0–10 cm using a spade from one of the inner three wheat rows (from a total of 7 wheat rows). Loosely attached soil was shaken off from the excavated root system before cutting off and transferring roots with the remaining rhizosphere soil into 50 ml tubes for further processing in the laboratory and molecular analyses. All samples were stored in cooling boxes, transferred to a cold room at 4 °C for overnight storage and processed the next day.

At maturity (July 24, 2019 and July 9, 2020), grains and straw were harvested from the central harvest plot (inner five wheat rows with 8 m length) using a plot harvester. The straw yield was directly weighed in the field and subsamples taken to the lab for further analyses, while grain yield and thousand-grain weight were assessed after cleaning and air drying.

2.3. Soil and plant-related analyses

Soil samples were sieved at 2 mm and either stored at 4 °C or air-dried and kept at room temperature for further analyses. Soil dry matter content was determined after drying fresh soil at 105 °C to constant weight. Soil pH was measured in water at a soil-to-water ratio of 1:2.5 (w/v), total soil C and N content (Ctot, Ntot) using an Elementar Vario Max Cube (Elementar Analysensysteme GmbH, Langenselbold,

Germany). A soil subsample was sent to a commercial laboratory (Labor für Boden- und Umweltanalytik, www.ericschweizer.ch) to determine P, K, Mg and Ca content in EDTA. Nmin was extracted the day after sampling using a 0.01 M $CaCl_2$ solution (1:4, w/v) before spectrophotometric determination (SAN-plus Segmented Flow Analyzer, Skalar Analytical B.V., AA Breda, The Netherlands) of nitrate and ammonium in the filtrate.

The 50 ml tubes containing the roots with the attached rhizosphere soil were shaken in 25 ml of sterile water for 3 min. Roots were removed and stored for further processing; the remaining soil-water suspension was centrifuged (10 min at 4000 x g). After decanting the supernatant, the sedimented rhizosphere soil was homogenised, lyophilized and stored in a desiccator until DNA extraction. Root samples were washed with tap water, surface sterilized by submerging them for 5 min in 2.5 % NaOCl enriched with one drop of Tween 20 (Carl Roth GmbH, Germany), washed three times in sterile water and dried (85 °C, 3 h). Cut roots were frozen at −80 °C, homogenized twice for 1.5 min in a TissueLyser (Qiagen, Hilden, Germany) at 30 Hz in two different orientations and stored at −20 °C.

Straw was dried for 48 h at 55 °C, shredded with a hand shredding device and milled in a hammer mill (SK 100, Retsch, Germany). Grains were air-dried, cleaned, weighed, and milled with a centrifugal mill (ZM200, Retsch, Germany). The C and N content in straw and grains was measured with a Vario Max Cube (Elementar Analysensysteme GmbH, Langenselbold, Germany).

2.4. DNA extraction, qPCR, amplicon sequencing and data processing

DNA was extracted from the lyophilized root (50 ± 5 mg) and rhizosphere (200 ± 50 mg) sample using the Qiagen DNeasy PowerSoil HTP 96 Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. Negative controls (DNA extraction blanks) were included. DNA concentration was measured using the Qubit fluorometric assay (Thermo Fisher Scientific, Waltham, USA) and extracts were stored at −20 °C.

Bacterial amplicon libraries were prepared in a two-step PCR approach according to the standard Illumina protocol. The primers used for the first PCR were 799f-Illumina and 1175r-Illumina (Bonder et al., 2012; Chelius and Triplett, 2001). Cycling conditions, oligonucleotide sequences and reference are listed in Table A2. Each sample was processed in technical triplicates to minimize stochastic PCR effects of individual reactions and pooled after PCR amplification. Fungal amplicon libraries were prepared in a three-step PCR approach, including technical triplicates per sample. For the first PCR, the primer mixtures SSUmAf and LSUmAr described by Krüger et al. (2009) predominantly targeting AMF, were used. PCR products were directly used as templates for a nested PCR using the modified forward primer LSUD2mod (Senés-Guerrero et al., 2020) and the LSUmBr reverse primer mix (Krüger et al., 2009) with attached Nextera overhang adapters for Illumina MiSeq. Cycling conditions, oligonucleotide sequences and reference are listed in Table A2. Amplicons were visualized on a 2 % agarose gel, before pooling and purification using AMPure XP beads (Qiagen, Hilden, Germany). In the third PCR, we indexed each sample with primers of the Nextera XT Index Kit (Illumina, Inc, USA). The bacterial and fungal libraries were sequenced on an Illumina MiSeq at the Competence Unit Bioresources of the AIT Austrian Institute of Technology in Tulln, Austria. The raw sequencing data were uploaded to the Sequence Read Archive (SRA) from NCBI (https://www.ncbi.nlm.nih.gov/sra/docs) under the project number PRJNA1157191.

The bioinformatics analysis was conducted at the Scientific Computer Cluster Euler at ETH Zurich. USEARCH v11.0.667 (Edgar, 2010) was used to remove phiX and merge read pairs with a minimum overlap of 30 bp and minimum merge length of 100 bp. Primer sequences were removed, and paired reads were size-selected, quality-filtered and denoised using USEARCH v11.0.667 (Edgar, 2010). Removal of chimaera and clustering into zero radius operational taxonomic units (ZOTUs) was done via UNOISE (Edgar, 2016b). Additionally, clustering at 97 % sequence identity was done by UPARSE (Edgar, 2013). Taxonomy was assigned via SINTAX (Edgar, 2016a) using SILVA v128 (Quast et al., 2012), UNITE_v82 (Bengtsson-Palme et al., 2013) as reference for the bacterial and fungal datasets at 0.85 and 0.5 tax filter identity threshold, respectively.

2.5. Data handling, analysis and visualisation

RSTUDIO (Prosit team, 2023), a development environment for R VERSION 4.3.0 (2023–04–21) was used to analyse the data (R Core Team, 2023). All graphs were created using the R package GGPLOT2 (Wickham and Wickham, 2016). To analyse the performance of the three wheat varieties in the different tillage and fertilization treatments, we fitted separate linear mixed-effect models for the years 2019 and 2020 using the lme4::lmer function (Bates et al., 2015). As fixed effects, we included the main experimental factors (tillage, fertilization intensity and variety), initially with all 2- and 3-way interactions involving the factor "variety", while the random part of the model accounted for the blocking design of the field by nesting the field "subplots" in the field "block".

After the initial model inspection, the decision regarding the inclusion of interactions in the model was guided by both theoretical considerations and statistical assessment. Upon the initial inclusion of interactions, we assessed their statistical significance through model summaries. Interaction terms that were found to be non-significant were subsequently removed from the model. This simplification aimed to enhance model interpretability, reduce complexity, and ensure a more parsimonious representation of the relationships among the studied variables. This way, we ensured that the final models retained adequate statistical power while maintaining a balance between complexity and interpretability. We followed this approach consistently in all models constructed in this study. After the final model was constructed, the model's fit was visually verified, and if necessary, data was transformed to conform to the model residuals' variance homogeneity and normal distribution assumptions. The ANOVA function was used to assess the statistical significance of the main effects and the emmeans::emmeans function (Russel et al., 2020) to provide estimated marginal means and 95 % confidence intervals for the experimental factors. To quantify differences between levels of the factors that explained a significant amount of variation in the data, we used the emmeans::emmeans function with the "Bonferroni" method to adjust for multiple comparisons. For each comparison, we calculate Cohen's d, providing a standardized measure of the difference between the group means using the emmeans::eff_size function with pooled standard deviation.

Chloroplast, mitochondria and cyanobacteria sequences were removed from the 16S rRNA amplicon datasets. Although archaea are important components of the microbial community in agricultural soils, we removed any sequences assigned to Archaea and refrained from in-depth analyses of those sequences since more tailored primers are available to target Archaea specifically. Fungal sequencing data were cleared from any sequence not assigned to the fungal kingdom. For all sequencing data, we generated rarefaction curves and abundance frequency plots (Figures A3, A4, A5, A6) to select appropriate rarefied library sizes for the downstream analysis. We then rarefied the bacterial and fungal data 500 times each to obtain equal library sizes in all samples and to reduce the uncertainty introduced through the random subsampling during rarefying. The resulting data was then averaged across the 500 rarefied libraries and used to calculate the OTU richness as the number of species, OTU diversity as the exponential of Shannon index (Jost, 2007) and Sheldon's evenness as $S = \frac{e^H}{N}$ with H representing the Shannon diversity and N the species number. The effects of the experimental factors were assessed as described above for the agronomic data.

To analyse treatment effects on beta diversity, low prevalent OTUs

were removed using filtering thresholds derived from exploring sequence prevalence graphically (Figure A5 and A6). For bacterial data, we removed sequences that occurred in less than 10 % of samples with less than a total of 100 sequences. For the fungal data, we split the rhizosphere and root samples and removed sequences that occurred in less than 10 % of samples with less than a total of 100 sequences for the rhizosphere and 5 % and 50 sequences for the root samples. Details on the resulting number of obtained sequences and associated OTUs after filtering are summarised in Table A3. We transferred sequence counts into relative abundances by total sum scaling before analysing the effect of tillage, fertilization, and variety and by including all interactions involving "variety" on the microbial community composition (beta diversity) with permutational multivariate analyses of variance (PERMANOVA) using *vegan::adonis2* (Oksanen et al., 2018) and Bray–Curtis distances. As the compartment (root vs rhizosphere in the case of fungi) and the year of sampling were the most influential drivers shaping the microbial community composition, we fitted separate models for the different years and, in the case of fungi, also for the root and rhizosphere samples. Regarding the inclusion of interactions, we followed the procedure described above for the univariate data.

Since differences in the multivariate spread can confound the results of the PERMANOVA test, we confirmed that the dispersion did not differ in the treatment groups by employing the functions *vegan::betadisp* and *vegan::anova* (Oksanen et al., 2018). To visually display the structures of bacterial and fungal communities, we used a nonmetric multidimensional scaling (NMDS) approach with Bray–Curtis distances, specifying a configuration of three dimensions (k = 3), and a maximum of 100 attempts (trymax = 100) in the vegan::metaMDS call (Oksanen J et al., 2022).

3. Results

3.1. Agronomic data

To explore potential candidate varieties for sustainable farming practices, the performance of three distinct wheat varieties was evaluated under both reduced and conventional tillage methods, coupled with varying levels of fertilization. For the response variables measured, we found no significant interactions between variety and tillage or varieties and fertilization, thus, interactions were excluded from the model to facilitate the interpretation and to prevent overfitting.

Across all experimental factors, the average straw yield was 8.19 t DM/ha (interquartile range (IQR): 1.65) in 2019 and 7.55 t DM/ha (IQR: 1.35) in 2020. In 2019, straw yield differed between the three wheat varieties with significantly higher straw yields in Wiwa when compared to Allez-y (+1.37 t DM/ha, Cohen's d=1.04). In 2020, the straw yields were affected by the fertilization treatment and wheat varieties: straw yields were higher under full vs half fertilization (+0.84 t DM/ha, Cohen's d=0.96). The variety Wiwa produced significantly higher straw yields as compared to Allez-y (+1.34 t DM/ha, Cohen's d=1.52) and Apache (+1.44 t DM/ha, Cohen's d=1.64), while results between the latter two varieties did not differ statistically (Fig. 1A, Table A4).

Across all experimental factors, the mean grain yield averaged 4.38 t DM/ha (IQR: 1.36) in 2019 and 3.93 t DM/ha (IQR: 1.06) in 2020. In 2019, tillage system and varieties significantly influenced grain yield: CT resulted in higher yields compared to RT (+0.88 t DM/ha, Cohen's d=1.81). In 2019, significantly higher grain yields were found comparing Apache to Wiwa (+0.80 t DM/ha, Cohen's d=1.66) and Allez-y (+0.55 t DM/ha, Cohen's d=1.15), while Allez-y and Wiwa demonstrated similar grain yields. In 2020, grain yield differences were observed based on fertilization treatments and wheat varieties: full fertilization led to increased yields compared to half fertilization (+0.59 t DM/ha, Cohen's d=1.73). Apache produced significantly higher yields compared to both Wiwa (+1.42 t DM/ha, Cohen's d=4.14) and Allez-y (+0.54 t DM/ha, Cohen's d=1.58) (Fig. 1B, Table A4).

Across all experimental factors, the mean grain N percentage averaged 1.76 % (IQR: 0.33) in 2019 and 1.57 % (IQR: 0.48) in 2020. In 2019, grain N varied across all three factors: higher values were observed under CT compared to RT (+0.07 %, Cohen's d=1.32) and under full fertilization versus half fertilization (+0.11 %, Cohen's d=2.01). Significantly higher grain N contents were found comparing Wiwa to Allez-y (+0.27 %, Cohen's d=4.78) and Apache (+0.40 %, Cohen's d=7.04), while Allez-y surpassed Apache (+0.13 %, Cohen's d=2.25). In 2020, grain N percentage varied in response to tillage practices and varieties: lower values were observed under CT compared to RT (-0.05 % points, Cohen's d=-1.11). Wiwa demonstrated significantly higher values compared to both, Allez-y (+0.37 %, Cohen's d=7.90) and Apache (+0.54 %, Cohen's d=11.38), whereas Allez-y surpassed Apache (+0.17 %, Cohen's d=3.48) (Fig. 1C, Table A4).

Across all experimental factors, the mean grain N uptake averaged 77.2 kg N/ha (IQR: 27.9) in 2019 and 60.1 kg N/ha (IQR: 11.6) in 2020. In both years, grain N uptake was influenced by fertilization and tillage treatments. Higher values were observed under full fertilization compared to half fertilization in 2019 (+13.1 kg N/ha, Cohen's d=1.38) and 2020 (+9.8 kg N/ha, Cohen's d=1.54), as well as under CT vs RT in 2019 (+19.0 kg N/ha, Cohen's d=2.00) and 2020 (+9.8 kg N/ha, Cohen's d=1.54) (Fig. 1D, Table A4).

Across all experimental factors, the average TWG measured 40.8 g/1000 kernels (IQR: 4.41) in 2019 and 44.5 g/1000 kernels (IQR: 4.28) in 2020. Variety significantly influenced TGW in both years. Allez-y displayed higher TGW than Apache in 2019 (+4.97 g/1000 kernels, Cohen's d= 3.06) and 2020 (+5.91 g/1000 kernels, Cohen's d= 5.12), while Wiwa exhibited higher TGW than Apache in 2019 (+3.96 g/1000 kernels, Cohen's d= 2.44) and 2020 (+4.20 g/1000 kernels, Cohen's d= 3.64). Additionally, in 2020 but not 2019, Allez-y values surpassed Wiwa (+1.71 g/1000 kernels, Cohen's d= 1.49) (Fig. 1E, Table A4).

3.2. Sequencing data

To assess the impact of tillage practice and fertilization intensity on microbial communities in organic systems, changes in diversity and community structure were investigated. After pre-processing as described in the methods, we detected a total of 5'072'205 (average: 52'835 sequences, median: 49'322, range: 9'581–126'840) bacterial sequences in the rhizosphere samples, translating into 14'597 unique OTUs. A total of 1.14 % of these sequences could not be assigned to a bacterial phylum. Averaged over the experimental treatments and years, the five most abundant bacterial phyla in terms of total abundance were identified as Proteobacteria, Actinobacteria, Bacteroidetes, Acidobacteria and Gemmatimonadetes. No difference in the taxonomic composition at the phylum level was observed between the treatments and across cropping seasons (Fig. 2).

Fungal communities, sequenced with primers originally designed to target AMF, were presented by 1'780'470 sequences in rhizosphere (average: 18'854, median: 16'520, range: 2'613–50'325) and 2'425'576 sequences in root samples (average: 25'266, median: 23'922, range: 24–61'884) translating into 491 OTUs in the rhizosphere and 266 OTUs in the roots, respectively. Although the primers were designed to target only Glomeromycota, also other fungi were sequenced. Comparing the taxonomic composition of fungal communities in terms of their relative abundance in the two cropping seasons, we observed an increase in Glomeromycota in the rhizosphere in 2020 compared to 2019 and a shift from Ascomycota to Basidiomycota from 2019 to 2020 in the roots (Fig. 3).

3.2.1. Alpha diversity

After the initial data exploration, we used the rarefied (N=500) data to calculate three alpha diversity indices to describe the impact of our experimental factors on the within-sample diversity. Since our data did not provide any evidence to support the presence of interactions between the experimental factors, in the interest of simplicity and clearer

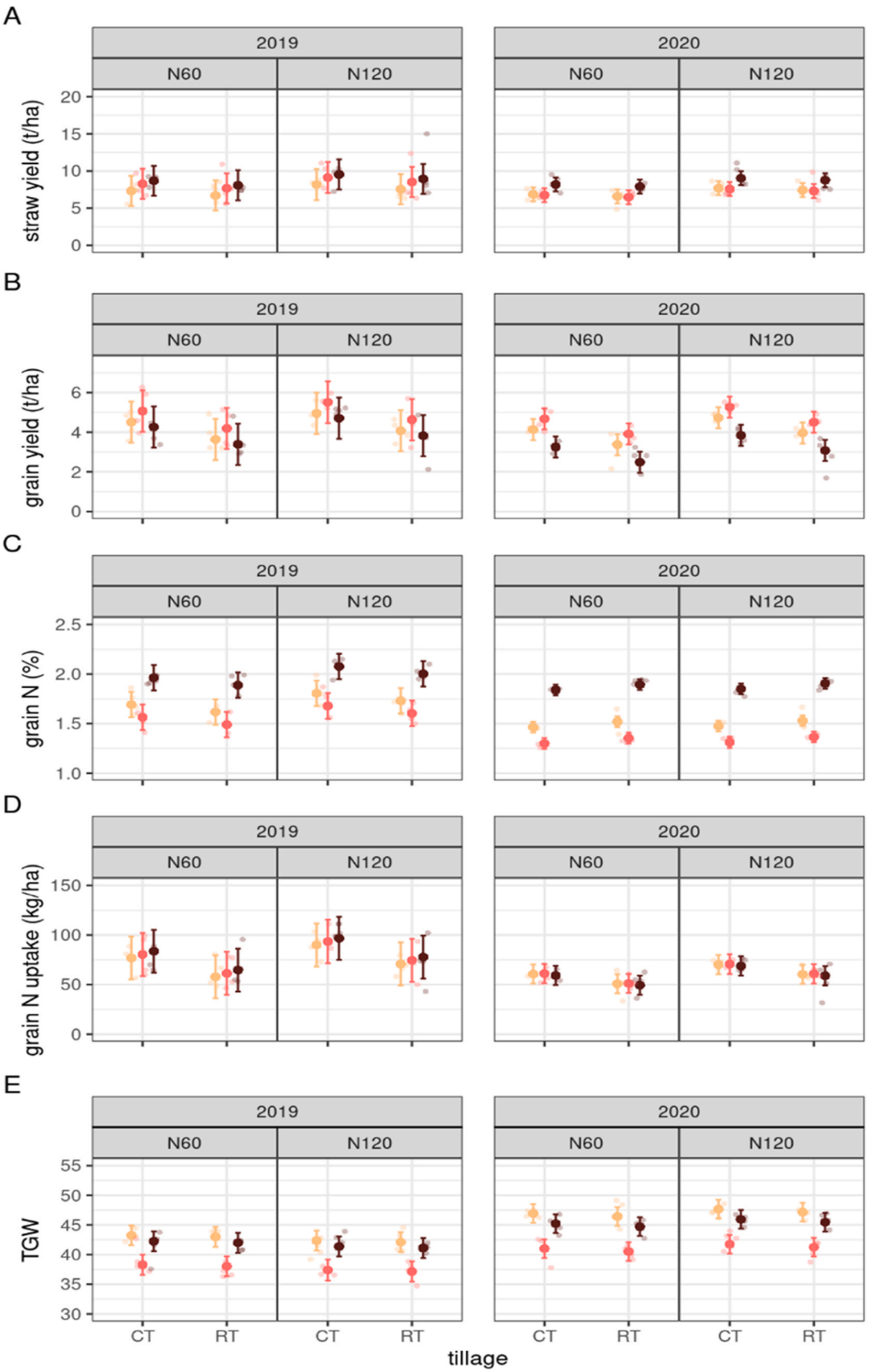

Fig. 1. Effect of tillage practices and Fertilization intensity on straw (A); grain yield (B); grain nitrogen (N) concentration (C); grain N uptake (D); and thousand grain weight (TGW, in g/1000 kernels) (E) as assed for the three wheat varieties Allez-y (yellow), Apache (orange) and Wiwa (brown). Marginal means with 95 % confidence intervals are represented with point and error bar and raw data are shown in lighter shade. CT: conventional tillage, RT: reduced tillage; N60: half fertilization; N120: full fertilization.

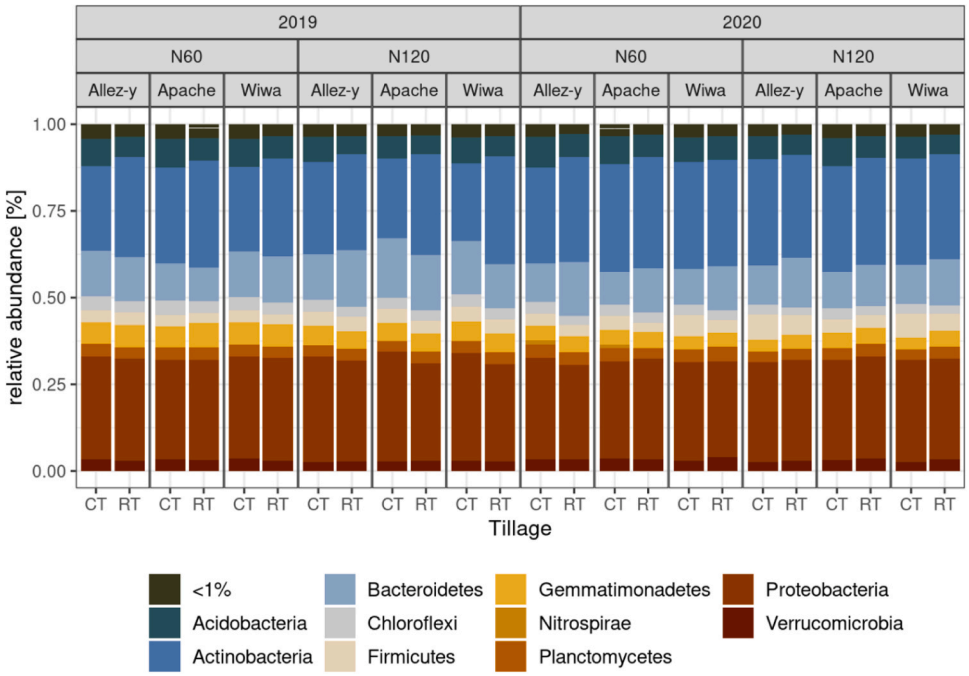

Fig. 2. Taxonomic composition of microbial communities after filtering low abundant OTUs. Bars show mean relative abundance across the field replicates (n=4). CT: conventional tillage, RT: reduced tillage; N60: half fertilization; N120: full fertilization. Taxa with abundance <1 % are grouped as a separate group.

interpretation, interactions were removed from the model.

Bacterial alpha diversity estimates were not affected by the experimental treatments in 2019 (Table A5, Fig. 4). In 2020, tillage affected bacterial OTU diversity and evenness: compared to CT, RT increased bacterial OTU diversity (+65.9, Cohen's d= 0.591) and evenness (+0.02, Cohen's d= 0.806) (Table A5, Figure A7). Fungal diversity and evenness in the rhizosphere during 2019 were affected by the factor tillage: CT exhibited higher fungal diversity compared to RT (+6.47, Cohen's d = 0.571), alongside greater evenness (+0.05, Cohen's d = 0.97) (Table A5, Fig. 4). In 2020, none of the experimental factors influenced alpha diversity estimates in the rhizosphere (Table S5, Figure A7). In root samples of 2019, we observed effects of fertilization on diversity and richness, with half fertilization resulting in higher diversity (+2.25, Cohen's d = 0.718) and richness (+6.57, Cohen's d = 0.697) compared to full fertilization (Table A5, Fig. 4). In 2020, effects of tillage on fungal OTU diversity and richness were noted in the root samples, with RT increasing diversity (+3.04, Cohen's d= 0.60) and richness (+8.85, Cohen's d= 0.65) compared to CT (Table SA5, Figure A7).

3.2.2. Beta diversity

The PERMANOVA analysis conducted on bacterial samples in the two years did not yield evidence supporting the presence of interactions between the experimental factors. As before, interactions were excluded from the model. In 2019, variations in bacterial communities within the rhizosphere were significantly influenced by tillage and fertilization, while the different wheat varieties did not exert a discernible impact during this period (Table A6A, Fig. 5A). In 2020, the composition of bacterial communities appeared to be predominantly shaped by the tillage factor (Table A6A, Fig. 5B).

As for bacteria, fungal community composition within the rhizosphere in the year 2019 was significantly influenced by the factors of tillage and fertilization, while the different wheat varieties did again not exert a discernible impact during this period (Table A6B, Fig. 5C). In 2020, the composition of fungal communities in the rhizosphere appeared to be predominantly shaped by the tillage factor (Table A6B, Fig. 5C). In the root samples of both years, we found fungal communities to be affected by the fertilization treatments only (Table A6B, Fig. 5D and E).

4. Discussion

4.1. Wheat variety most consistently influenced agronomic traits in winter wheat

Amongst all the factors investigated, wheat variety was the most important factor affecting wheat growth and N uptake in both years. While the variety Wiwa achieved the highest straw yields and grain N percentages paired with the lowest grain yields, Apache produced the highest grain yields but the lowest N percentages. This observed negative correlation between grain yield and grain N percentage is a recognized trade-off (Hawkesford and Riche, 2020), likely attributed to distinct nutrient foraging strategies among different crops (Hawkesford and Riche, 2020; Lollato et al., 2021; Swarbreck et al., 2019). While we intentionally chose wheat varieties with divergent traits related to their susceptibility to biotic and abiotic stresses, we did not detect any interactions between fertilization intensity, tillage, and wheat variety. This finding underlines the challenge of breeding varieties that are able to maintain high yields with reduced fertilizer use (Hirel et al., 2011). Another critical point to consider is the pedoclimate-specific performance that wheat varieties may exhibit (Soofizada et al., 2023; Wilkinson et al., 2023). However, Wilkinson et al. (2023) have demonstrated that the performance of wheat varieties can also remain stable under similar pedo-climatic conditions. This finding emphasizes the necessity of evaluating wheat varieties under contrasting pedo-climatic conditions to identify those that are optimally suited for cultivation within specific agricultural systems.

Concerning the influence of management practices, we noted elevated grain yields and grain N uptake in CT compared to RT in both cropping seasons. Furthermore, there was a consistent increase in grain N uptake under full fertilization compared to half fertilization in both cropping seasons. Our findings are consistent with the meta-analysis of Cooper et al. (2016), reporting a 7 % yield reduction in organic reduced tillage systems compared to ploughed systems. However, the magnitude of yield decrease under RT vs CT varies across studies, ranging from 15 % to no loss or even higher yields under RT conditions in some cases

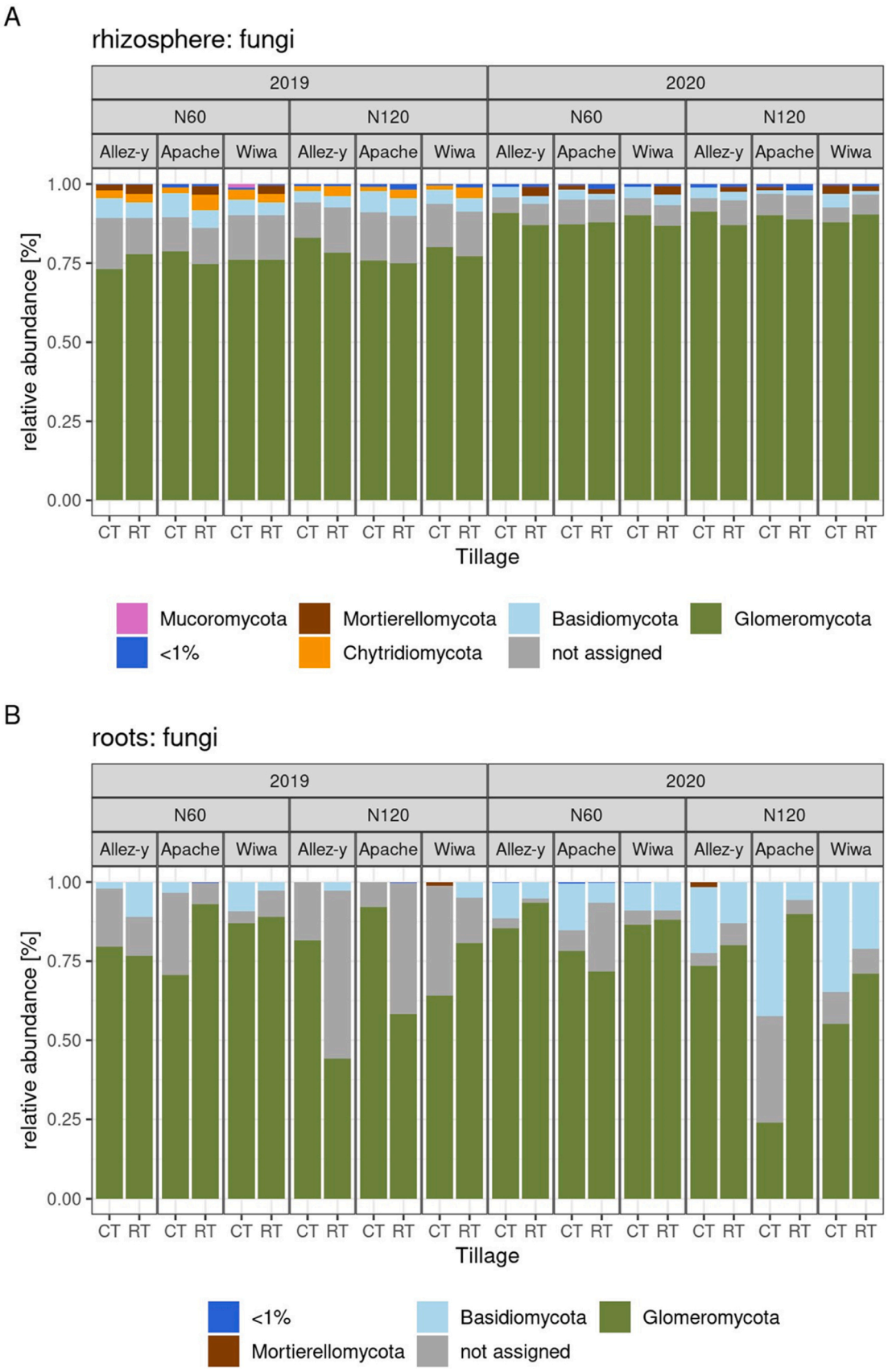

Fig. 3. Taxonomic composition of microbial communities after filtering low abundant OTUs as described in the method section. Bars show mean relative abundance across the field replicates (n=4). CT: conventional tillage, RT: reduced tillage; N60: half fertilization; N120: full fertilization. Taxa with abundance <1 % are grouped as a separate group.

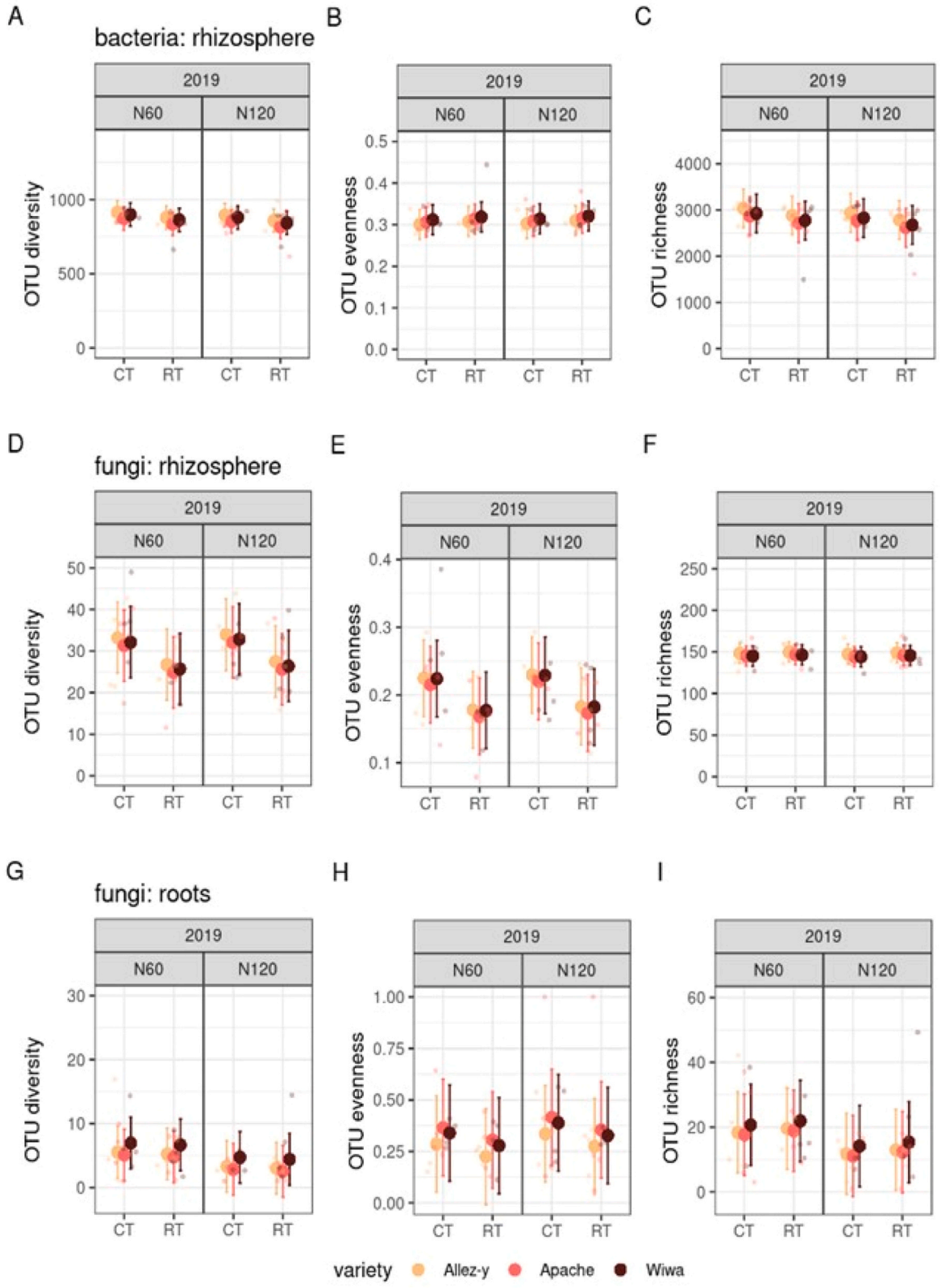

Fig. 4. Effects of tillage and fertilization on operational taxonomic units (OUT) Shannon diversity of bacterial communities in the rhizosphere (A), fungal communities in the rhizosphere (D), fungal communities in the roots (G), Sheldon's evenness of bacterial communities in the rhizosphere (B), fungal communities in the rhizosphere (E), fungal communities in the roots (H), OTU richness of bacterial communities in the rhizosphere (C), fungal communities in the rhizosphere (E), fungal communities in the roots (I) in samples collected in 2019. Diversity of samples collected in 2020 are shown in Fig. A7). CT: conventional tillage, RT: reduced tillage; N60: half fertilization; N120: full fertilization.

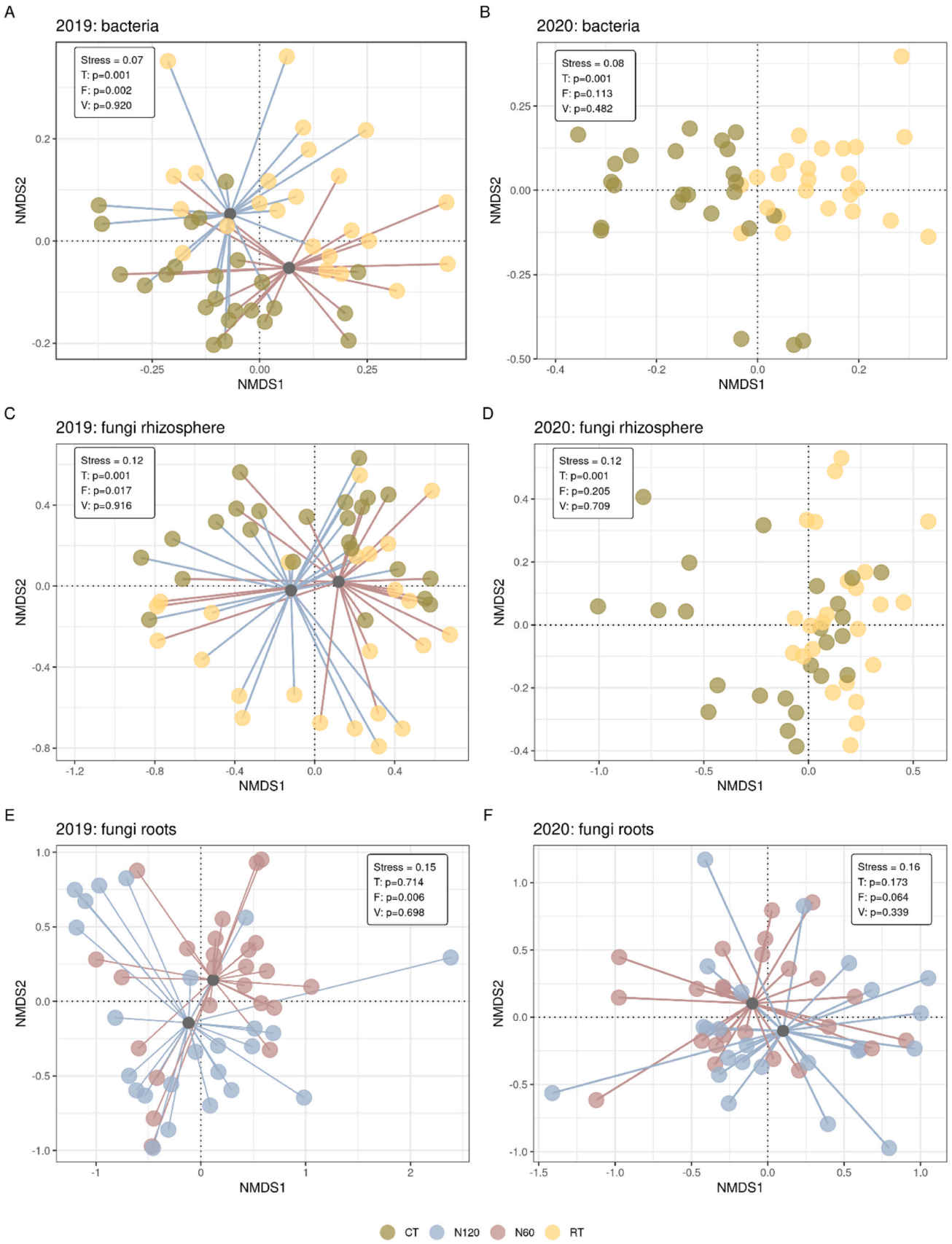

Fig. 5. NMDS of bacterial communities in the rhizosphere in (A) 2019 and (B) 2020 and fungal communities in the rhizosphere in 2019 (C) and 2020 (D) and fungal communities in root samples of 2019 (E) and 2020 (F). The p-values of the PERMANOVA function are provided in each figure along with the stress value representing the accuracy of data representation in lower dimensions compared to the original higher-dimensional space. T: Tillage (CT: conventional tillage, RT: reduced tillage); F: Fertilization (N60: half fertilization; N120: full fertilization) and V: Variety (WIWA, Allez-y, Apache). A significant fertilization effect is presented with centroids.

(Büchi et al., 2017; Fan et al., 2012; Głąb and Kulig, 2008; Hofmeijer et al., 2019; Krauss et al., 2020; Peigné et al., 2014; Tørresen et al., 2003). These studies highlight the importance of the local pedo-climatic conditions (Bücheli et al., 2017). Another factor potentially impairing wheat growth in Aesch might be the higher weed pressures observed under reduced tillage compared to ploughing. A meta-study by Cooper et al. (2016) also found that increased weed pressure and reduced N availability due to lower mineralization are main drivers limiting wheat yields under reduced tillage. Given that grain N percentage remained comparable under reduced and conventional tillage, we assume that the higher weed pressure and associated competition for resources under reduced vs conventional tillage could have been the primary limiting factor in our study. Also, Hofmeijer et al. (2019) observed that weed infestation mainly limited wheat performance under reduced tillage compared to ploughed systems. Removing weeds from the plots resulted in grain yield increases only under reduced tillage, while grain yields were constant in ploughed plots. In addition, it is anticipated that the yield gap between conventional and reduced tillage will diminish progressively over time. This expectation arises from the fact that the advantageous impacts of reduced tillage such as increasing the organic matter reservoir in the soil, may require a timeframe of 10–20 years to manifest fully (Büchi et al., 2017), whereas the beneficial effects of conventional tillage (i.e. increased mineralisation) occur within a matter of weeks.

4.2. Management practices but not wheat variety were the main drivers for changes in microbial community composition

Across both cropping seasons, none of the investigated factors consistently impacted fungal and bacterial alpha diversity. While in the rhizosphere, bacterial diversity was increased under RT compared to CT only in 2020, fungal diversity was increased in CT compared to RT only in 2019. Root fungal communities were increased due to full fertilization in 2019 and under RT compared to CT in 2020. Similarly, in a meta-study, de Graaff et al. (2019) found that the effects of tillage on soil microbial diversity were highly variable and mainly dependent on climatic conditions and soil properties. The significant differences in temperature and precipitation patterns, reflected in the different soil moisture contents at the time of sampling in the two growing seasons, may account for the observed inconsistent patterns in microbial alpha diversity. In 2019, spring (March to May) was wetter (179 mm precipitation) and slightly colder (mean temperature 8.6 °C) compared to 2020, which was drier (135 mm precipitation) and warmer (mean temperature 9.9 °C). This suggests that management practices may play a limited impact compared to other factors, particularly climatic conditions, which seem to exert a greater influence on bacterial and fungal alpha diversity. Notably, this study is one of the first to analyse the diversity of microbial communities at the same crop stage over two growing seasons. Previous research in this area has mainly focused on communities sampled in a single season, neglecting the considerable variability between growing seasons. In a similar context, temporal changes in bacterial and fungal alpha diversity have been observed over the course of a growing season. The study showed that the effects of management practices were not consistent at each sampling time point (Fox et al., 2022).

On the contrary, microbial community composition (beta diversity) was significantly and consistently influenced by agricultural management practices in both years. This consistency in the effects on community composition compared to the effects on diversity aligns with previous research showing more robust and persistent effects of agronomic practices on the composition of bacterial and fungal communities compared to their effects on microbial diversity (Hartmann and Widmer, 2006). Microbial community composition changes may not always result in diversity or richness alterations, as shifts in some taxonomic groups can be offset by changes in others, thereby maintaining overall diversity levels despite fluctuations in composition.

The current study found a persistent effect of tillage on both bacterial and fungal communities in the rhizosphere over both cropping seasons. This is in line with previous studies reporting about differences in microbial community structure between ploughed systems compared to reduced tillage and even more, no-tillage systems (Hartman et al., 2018; Kraut-Cohen et al., 2020; Orrù et al., 2021; Wang et al., 2020). One of the main drivers for the observed differences might be the higher SOC content in RT compared to CT plots in the Aesch trial as also reported by Grosse et al. (in prep.). Increased SOC is positively associated with higher microbial community size and activity, as shown in the meta-study by Lori et al. (2017), and has been described as a main driver for changes in microbial communities (Ramírez et al., 2020). Another driver for differences in microbial community structure might be the higher weed infestation in the RT compared to the CT plots in the Aesch trial. A higher aboveground diversity has already been reported to be an important driver for changes in microbial communities due to the higher number of potential host plants and a higher variation of available root exudates (Zhalnina et al., 2018). Contrary to our hypothesis, tillage had no effect on AMF targeted-fungal root communities and, interestingly, few studies have investigated the effects of tillage on root communities. Similarly, Hartman et al. (2018) observed no effect of tillage on fungal root communities in winter wheat collected from a Swiss long-term tillage and cropping system comparison trial. A declining influence of treatment effects from the rhizosphere to the roots has been described in a few other studies. Hartman et al. (2018) observed effects of tillage and cropping systems on soil bacterial and fungal communities, but not in roots. Romano et al. (2023) also observed a decreasing effect of fertilization and tillage from the rhizosphere to root microbial communities, as did Wang et al. (2017) who observed a decrease in effects from the rhizosphere to root communities in experiments testing elevated CO_2 in maize and soybean. This decrease in management effect from rhizosphere to the roots might be explained by selective plant-root-microbe communication (Haldar and Sengupta, 2015; Lareen et al., 2016) which involves an increase in selectivity from the rhizosphere towards the roots firstly, by the attraction of soil microbes with root-colonizing traits via root exudates and secondly by differences in the ability of selected soil microbes to effectively colonize roots (Van der Heijden and Schlaeppi, 2015).

Interestingly, we observed that fertilization intensity only affected fungal and bacterial rhizosphere communities in 2019 and fungal root communities across both years. The direct and indirect effect of N availability, and N fertilization in general, on microbial community structure has been described earlier, partly due to a shift towards a more copiotrophic microbial community (Li et al., 2021; Liu et al., 2021; Ramirez et al., 2010). The discrepancy in observed effects between the first and second year could be attributed to environmental conditions such as the lower soil water content in the 2019 compared to 2020. This difference may have led to reduced N availability, resulting in a greater disparity between the two fertilization intensities.

The distinction between the growing seasons was also evident when comparing the relative abundances of fungal genera. In the rhizosphere, we detected a higher relative abundance of species belonging to Glomeromycota in 2020 compared to 2019. This difference might have resulted from the additional cycle of maize as main crop preceded by *Brassica rapa* as cover crop on 2020 vs 2019 plots (Figure S1). Maize is a well-known host for AMF and is often used for their mass propagation (Sharma et al., 2017). As a C4 plant with elevated photosynthetic rates (von Caemmerer and Furbank, 2016), maize, similar to sorghum, efficiently supplies carbon to the AMF network, which may help increase AMF presence in soils (Walder et al., 2016). Analyzing AMF colonization in wheat roots through traditional staining and microscopy techniques would have provided valuable complementary data to the sequencing results. This approach could offer deeper insights into the effects of incorporating an additional cycle of maize or an alternative C4 crop into the cropping sequence, potentially enhancing AMF colonization rates in wheat.

5. Conclusion

Our study underlines the predominance of wheat variety as the main determinant of both yield and yield quality traits, irrespective of tillage and fertilization practices. This suggests the need for intensified breeding efforts focused on developing wheat varieties with enhanced resilience to stressful conditions, such as nutrient limitations and waterlogging probably resulting from an increase in soil density in the former plough layer. Such efforts should prioritize traits that improve root architecture, nutrient uptake efficiency, and tolerance to abiotic stressors, enabling these varieties to thrive in challenging environments. In addition, our results highlight the need for tailored and adaptive management strategies to mitigate the challenges associated with tillage-induced constraints such as reduced nutrient availability and increased soil densities of former plough layers. These strategies are critical to maintain consistent crop performance in reduced tillage wheat systems, ensuring sustainable productivity while minimizing the negative effects of tillage practices. Furthermore, our microbiome analyses reveal that wheat variety does not significantly influence microbial community composition. To the contrary, we observe contrasting effects of management practices on rhizosphere and root-associated microbial communities, which is particularly notable when implementing practices that promote the colonisation of beneficial root symbionts.

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CRediT authorship contribution statement

Friederike Trogniz: Writing – review & editing, Supervision, Resources, Formal analysis, Conceptualization. **Dominika Kundel:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Formal analysis, Data curation. **Marcé Doubell:** Writing – review & editing, Resources, Methodology, Formal analysis, Data curation. **Hanna Faist:** Resources, Methodology, Investigation, Formal analysis, Data curation. **Angela Sessitsch:** Writing – review & editing, Supervision, Resources, Funding acquisition, Conceptualization. **Sarah Symanczik:** Writing – review & editing, Writing – original draft, Supervision, Resources, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Maike Krauss:** Writing – review & editing, Supervision, Resources, Investigation. **Natacha Bodenhausen:** Writing – review & editing, Methodology, Conceptualization. **Stéphane Declerck:** Writing – review & editing, Resources, Methodology, Investigation, Formal analysis, Data curation.

Declaration of Competing Interest

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

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.still.2024.106328.

Data Availability

All data are available online on Zenodo (doi.org/10.5281/zenodo.13751649) and on the Sequence Read Archive (SRA) from NCBI under the project number PRJNA1157191.

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